VITAMIN D, COGNITIVE FUNCTION, AND OXIDATIVE STRESS: CLUES TO OVERTRAINING SYNDROME?

By

Scott P. Jerome, B.A., M.S.

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APPROVED:

Dr. Arleigh J. Reynolds, Committee Chair
Dr. Lawrence K. Duffy, Committee Member
Dr. Daní K. Sheppard, Committee Member
Dr. Phillip B. Watts, Committee Member
Dr. Thomas K. Green, Chair
Department of Chemistry & Biochemistry
Dr. Anupma Prakash, Interim Dean
College of Natural Science & Mathematics
Dr. Michael A. Castellini, Dean
Graduate School

Abstract

Overtraining syndrome (OTS) is characterized by an unexplainable drop in athletic performance. It affects primarily elite, endurance athletes, though sub-elite athletes are also affected. Although the deterioration in performance is often the most pronounced and troublesome symptoms for athletes, others range from severe fatigue and insomnia to depression and lack of mental concentration. There is no known diagnostic tool except for ruling out all other possible explanations for the abnormal performance. The only known remedy for OTS is rest. Some recover within months while others take a year or more. Some athletes never fully recovery and never return to pre-OTS performance levels.

The exact mechanism behind OTS is unknown. Consensus has been reached among exercise science professionals that 1) an imbalance between stress load and recovery leads to OTS; 2) OTS exists on a spectrum of possible outcomes from different exercise/rest ratios; and 3) exercise is only one part of systemic stress that can lead to OTS. In addition to physical exercise, other factors such as environmental conditions, family dynamics, schoolwork, job stressors, and social pressures all contribute to the total stress load on the body. A severe and sustained imbalance between stress and rest is a likely contributor to OTS in athletes.

I investigated biomarkers and psychological markers that, in concert, could be used to identify athletes who are at the greatest risk for developing OTS before the onset of symptoms. I examined vitamin D, cognitive function, and oxidative stress status in university cross country skiers in addition to athletic performance status during the competitive ski season. This study's results support three primary conclusions. First, collegiate endurance athletes are more prone to vitamin D insufficiency and deficiency than their sedentary counterparts. Second, collegiate

cross country ski racers in the circumpolar North are unlikely to maintain adequate vitamin D during a competition season. Furthermore, vitamin D levels are likely to drop in the post-season, recovery period. Third, cognitive function is likely to be significantly higher in the post-season than during the competition season. Fourth, those who experienced a drop in performance during the competition season are more likely to show signs of oxidative stress. These findings may help to produce a screening tool for OTS.

Dedication

This paper is dedicated to those looking for a second act. May we all find one.

Table of Contents

List of Figures

Page

List of Tables

Page

General Introduction

The human body is able to respond to a stress response through adaptation and/or diversification by means of gene expression [1]. This adaptation to stimuli is, at the most basic level, the essence of physical exercise. When homeostasis is disrupted subsequent adaptation occurs. Exercise ("athletic training" or "training") is based on this very principal: the body will adapt to stress to reach a new level of fitness – a new state of homeostasis. Whether it is a child who decides to run to the market one day instead of walking, or a world class sprinter performing squat exercises with higher resistance, adaptation to physical stimuli is the nature of exercise and sport. Indeed, the motto of the modern Olympic Games, "Citius, Altius, Fortius", captures the premise that the body can adapt to exercise and reach new levels of performance: "Faster, Higher, Stronger".

The process of stress and adaptation is not complete, however, without an adequate "resting" period or "recovery" state between exercise bouts [2]. The terms "rest and "recovery" may refer to several distinct conditions and processes. Recovery may indicate time between training sessions, specific physiological processes or conditions that are different than those found during exercise, or a cue indicating when exercise may resume again [2]. "Physiology of recovery", a sub-discipline of exercise physiology, focuses on the period of time between the cessation of one bout of exercise and the commencement of the next [2]. While the field is evolving, one constant remains clear. Without adequate recovery from exercise the body will not adapt [3], making recovery as important for improved athletic performance as exercise itself.

Training and recovery are meshed together on a spectrum of possible, exercise-related outcomes. Hallmarks of this spectrum include functional overreaching (FO), non-functional overreaching (NFO), and overtraining (OT). FO is the normal overload, recovery, and super-

compensation cycle required for improvement in athletic performance. NFO shares an overload feature with FO, but an ineffectual recovery period leads to stagnant performance. OT shares an overload period and ineffectual recovery with NFO. However, instead of a performance plateau, athletic performance declines with OT [4]. If OT persists, an athlete may succumb to overtraining syndrome (OTS).

OTS is characterized by an otherwise unexplainable drop in athletic performance. OTS affects both the sympathetic and parasympathetic nervous systems [5]. In addition to the decline in athletic performance, symptoms range from depression and bradycardia to insomnia and tachycardia (Table I.1). There is no universal diagnostic tool for OTS and many OTS symptoms can be caused by other factors such as endocrinological disorders, eating disorders, anemia, viral infections, and bacterial infections [4]. As such, a clinician must rule out these other possibilities for the decline in performance before considering OTS.

An athlete may experience NFO, OT, and OTS as he/she trains according to a comprehensive exercise plan that has worked in the past and/or works for other athletes of similar abilities. A plateau in performance and/or a drop in performance is not necessarily associated with a substantial increase in training load. Recovery is as important to increased athletic performance as exercise itself; therefore, some researchers and physicians refer to OTS as "underperformance syndrome" or "unexplained underperformance syndrome" (UUS) [6]. It is quite likely that the body which is not permitted to reach homeostasis before undergoing another exercise bout will not adapt effectively to the new stress. A disrupted homeostasis may have as much to do with factors outside of training – family, school, relationships, work – than with the actual physical stress of exercise [6].

The most agreed upon treatment for OTS is rest, either Relative (some low intensity bouts of exercise) or Absolute (no exercise or other strenuous, physical exertion) depending on the severity [6]. Some evidence suggests that a high carbohydrate diet may prevent development of OTS, and speed recovery from OTS [4]. Other researchers have suggested that, owing to the similarities of neuroendocrinologic changes between depression and OTS, that a selective serotonin reuptake inhibitor (SSRI) may be an effective OTS treatment [5]. However, there is a danger of the development of heat stress with an SSRI, as well as decreased athletic performance with antidepressant medications [5]. Furthermore, SSRI usage for OTS is not FDA approved [6]. Recovery for most OTS athletes takes months [4] while some never fully recover [6].

While the "cause" of OTS seems relatively simple – an imbalance between exercise and recovery – the actual etiology has yet to be pinpointed. Several hypotheses exist, ranging from glycogen depletion to cytokine-induced, systemic inflammation (Table I.2) [5].

In addition to an unknown etiology, there is no known method to determine which athletes are at an increased risk of developing OTS. The overarching goal of my PhD research is to pinpoint physiological biomarkers and/or psychological markers that will, either singly or in concert, help predict who is at the greatest risk of developing OTS prior to the onset of symptoms. Finding markers of OTS would, theoretically, allow trainers and athletes to adjust training, rest, and lifestyle in a purposeful way to maximize performance and minimize risk of OTS. The difficulty of diagnosis and the ethical concerns of inducing OTS make it challenging to study. I chose to focus on vitamin D deficiency (Chapter 1 and 2), cognitive function (Chapter 2), and the oxidative stress hypothesis (Chapter 3). Initially I studied vitamin D in active university athletes and compared them to their sedentary counterparts to establish whether exercise compounded the risk of insufficiency and deficiency in the circumpolar North (Chapter

1). After establishing the increased risk, I followed up with a second human subject study that monitored university athletes through training macrocycles to determine if biomarkers increased, decreased, or remained the same during the stressful competition season compared to one month of post-season recovery. Additional comparisons were made between participants who reported an unexplainable drop in performance lasting at least three weeks and those who did not.

Certain OTS symptoms, particularly a loss of mental concentration and muscle weakness, are similar to vitamin D deficiency symptoms. Most athletes with OTS (67%) have "total autonomic dystonia", a maladaptation associated with depressed regulatory function of the autonomic nervous system, both sympathetic and parasympathetic [7]. A lack of concentration and depression are common symptoms among OTS athletes [5]. Vitamin D deficiency is linked to depressive symptoms [8] and decreased cognitive function [9], though the latter is more pronounced in the elderly.

There are two primary avenues by which humans receive vitamin D: cutaneous synthetization and ingestion. The former is the major source of vitamin D for most humans [10]. Diet can also contribute significantly. Certain naturally occurring foods, primarily oily fish such as mackerel and salmon, are rich in vitamin D. Some fungi and plants offer limited amounts of vitamin D. Fortified foods such as dairy products and some fruit juices also contribute to vitamin D intake. Oral vitamin D supplements of at least 800 IUs per day should provide vitamin D adequacy unless there are mitigating factors [11]. While this is a seemingly straightforward recommendation, vitamin D deficiency has been recognized as a worldwide pandemic [10].

Biosynthesized from cholesterol, 7-dehydrocholesterol is the precursor to vitamin D. The sun's ultraviolet radiation converts 7-dehydrocholesterol in the skin to vitamin D_3

(cholecalciferol) which then undergoes two hydroxylations in the liver. First, D_3 is hydroxylated to $25(OH)D_3$, then to $1,25(OH)_2D_3$, the biologically active form of vitamin D [12].

Vitamin D_2 (ergocalciferol) is found mainly in plants and fungi. When ingested, D_2 can be converted for use, though it remains a relatively small source of naturally occurring vitamin D for most individuals. Vitamin D_2 is hydroxylated twice in the liver in a similar fashion as D_3. It is first converted to $25(OH)D_2$, then to $1,25(OH)_2D_2$. Both $1,25(OH)_2D_3$ and $1,25(OH)_2D_2$ have the same functionality. Vitamin D_2 and vitamin D_3 are considered bioequivalent and both can be used effectively in supplements [13].

Prevalence of vitamin D deficiency is greater at higher latitudes [14]. During the winter months at locations above 35° latitude the zenith angle of the sun is more oblique which prevents few, if any, UVB rays from reaching the earth's surface; the winter zenith angle of the sun severely limits or prevents vitamin D production in the skin [15]. High rates of vitamin D deficiency is also well documented among athletes [16, 17, 18]. Deficiency for many athletes follows a seasonal shift [16, 19, 20]. In a meta-analysis of 23 studies including 2,313 athletes, Farrokhyar et al. found 56% of athletes had vitamin D inadequacy that varied significantly by geographical location [21].

Considering the overlapping symptoms of OTS and vitamin D deficiency, vitamin D deficiency among athletes, and vitamin D deficiency in the circumpolar North, I chose to investigate vitamin D status as possible indicator of NFO and OT. Due to the implications of OTS and vitamin D deficiency on cognitive function, I also elected to examine basic hand-eye coordination in endurance athletes using the Purdue Pegboard Test (PPT).

The PPT is a simple test of cognitive function. The PPT involves three timed batteries in which participants place small metal pegs into holes. The sequence begins with the dominant

hand, then the non-dominant hand, and finally both hands together. A fourth timed battery requires the participant to build small assemblies using a peg, a washer, a collar, and a second washer. A study of the PPT's three trial, test-retest procedure demonstrated high reliability [22].

The oxidative stress hypothesis (OSH) of OTS contends that pathologic levels of oxidative stress can cause inflammation, muscle fatigue, and soreness which, in turn, results in a deterioration of athletic performance. In overtrained athletes, Tanskanen, Atalay, and Uusitalo found antioxidant protection against acute stress from exercise was impaired and resting oxidative stress increased [23]. In a study that overtrained humans for three weeks within a 12-week training cycle, Margonis et al. found that the high volume and intensity of exercise caused striking oxidative stress response [24]. Two markers of oxidative stress, catalase and glutathione peroxidase activity, increased significantly while total antioxidant capacity (TAC) dropped significantly. Although the OSH does not account for all OTS symptoms, the hypothesis is strong enough to justify further research. Therefore, I chose to examine markers of oxidative stress while, at the same time, surveying participants for relative athletic performance.

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Table I.1. Symptoms of OTS

Parasympathetic Alterations	Sympathetic Alterations	Other
Fatigue	Insomnia	Anorexia
Depression	Irritability	Weight loss
Bradycardia	Agitation	Lack of mental concentration
Loss of motivation	Tachycardia	Heavy, sore, stiff muscles
	Hypertension	Anxiety
	Restlessness	Awakening unrefreshed

Adapted from Kreher & Schwartz, 2012.

Table I.2. Recognized hypotheses of OTS etiology.

Hypothesis	Theory
Glycogen depletion	↓ glycogen leads to: ↑ fatigue ↓ performance
Central fatigue	↑ tryptophan uptake in brain leads to: ↑ serotonin centrally ↑ mood symptoms
Glutamine	↓ glutamine leads to: ↓ immune system function ↑ infection susceptibility
Oxidative stress	↑ oxidative stress leads to: ↑ fatigue ↑ muscle damage
Autonomic nervous system	↑ parasympathetic control causes many symptoms of OTS
Hypothalamic	Imbalance in the hypothalamus and hormonal axes cause many symptoms of OTS
Cytokine	Inflammation and cytokine activation can account for most OTS symptoms

Adapted from Kreher & Schwartz, 2012.

Chapter 1: 25(OH)D levels in trained v. sedentary university students at 64° north[1,2]

Abstract

Purpose – 25-hydroxyvitamin D (25[OH]D) deficiency is associated with compromised bone mineralisation, fatigue, suppressed immune function and unsatisfactory skeletal muscle recovery. We investigated the risk of 25(OH)D insufficiency or deficiency in endurance athletes compared to sedentary non-athletes living at 64° north.

Methods – University student-athletes (TS) and sedentary students (SS) volunteered to participate in this study. TS engaged in regular exercise while SS exercised no more than 20 minutes/week. Metabolic Equivalent of Task (MET) scores for participants were determined. Vitamin D intake was assessed using the National Cancer Institute's 24-hour food recall (ASA24). Fasting plasma 25(OH)D levels were quantified via enzyme-linked immunosorbent assay.

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[1] Scott P. Jerome[1], Kendra D. Sticka[1,2], Theresia M. Schnurr[1,6], Sally J. Mangum[3], Arleigh J. Reynolds[4], and Kriya L. Dunlap[1,5]

[1]Department of Chemistry and Biochemistry, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA. [2]College of Health, University of Alaska Anchorage, Anchorage, AK, 99508. [3]Pacific Northwest University of Health Sciences, Yakima, WA, 98901, USA. [4]Department of Veterinary Medicine, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA. [5]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA. [6]Novo Nordisk Research Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark.

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Results – TS reported higher activity levels than SS as assessed with MET-minutes/week and ranking of physical activity levels ($p<0.05$). The reported mean daily intake of vitamin D was higher in TS compared to SS ($p<0.05$) while 25(OH)D plasma levels were lower in TS than in SS ($p<0.05$). In total, 43.8% of the TS were either insufficient (31.3%) or deficient (12.5%) in 25(OH)D, while none of the SS were insufficient and 13.3% were deficient.

Conclusion – TS are at increased risk of 25(OH)D insufficiency or deficiency compared to their sedentary counterparts residing at the same latitude, despite higher vitamin D intake.

Introduction

Vitamin D insufficiency and deficiency in adults and children has been described as endemic worldwide [1, 2, 3, 4, 5] and investigated extensively in the recent past. One of the earliest scientifically identified roles of vitamin D supplementation is regulation of proper cellular calcium function in the prevention and treatment of rickets in children and osteomalacia in adults [6, 7]. In addition to these roles in mineral balance and bone metabolism, vitamin D has pleiotropic effects in many human cells [8]. While consensus has not been reached, recent evidence suggests that vitamin D may play a significant role in overall health in addition to bone strength and density. Health issues linked to insufficient or deficient levels of vitamin D include diabetes [8, 9], cancer [2], thyroid function [10], cardiac volume [11], immune-system function [12], obesity [13], cardiovascular disease and metabolic syndrome [8]. Though further research is needed, researchers have demonstrated that vitamin D sufficiency is positively correlated to proper muscle function [4, 14, 15, 16, 17] and skeletal muscle recovery and regeneration [18] while deficiency is associated with muscle pain, weakness [1] and inadequate repair [18].

A rising number of studies suggest that many athletes do not maintain sufficient levels of 25(OH)D [19] though few studies have investigated this circumstance with relation to geographical latitude [20, 21, 22]. The prevailing hypothesis denotes that small sun angles in winter months at latitudes greater than 42° result in limited or insubstantial conversion of 7-dehydrocholesterol to vitamin D_3 in the skin [1, 23, 24]. In a cross-sectional study of 2,548 adults in Norway, Larose et al described overall 25(OH)D deficiency (<50 nM) at 40% with a seasonal shift of 64% in winter to 20% in summer [3]. Vitamin D levels in populations living at high latitudes are more likely to be inadequate as compared to populations living near the equator [25, 26, 27]. These findings along with those suggesting that athletes are a greater risk of inadequacy

than non-athletes may prove to compound the overall risk of athletes living and training at high latitudes.

Our aim was to test the hypothesis that athletes living in Fairbanks, Alaska, at 64° N are at greater risk of vitamin D insufficiency and deficiency as compared to their sedentary counterparts living at the same geographic location.

Methods

Approval for this study was secured by the Institutional Review Board of the University of Alaska Fairbanks (UAF, #492213-4) prior to data collection. Following an explanation of the study, including risks and benefits, we obtained written consent from all participants.

A power analysis using previous studies with athletes was completed prior to recruitment of participants. Thirty-one male (n=16) and female (n=15) study participants, all non-pregnant and non-diabetic, 18-25 years of age, were recruited from the UAF student-body. They consisted of two groups: trained student-athletes (TS, n=16) and sedentary, non-athlete students (SS, n=15). All TS participants were members of the UAF cross country skiing and/or cross country running teams. TS were engaged in regular endurance exercise for 10 to 20 hours per week for at least three months prior to data collection in preparation for National Collegiate Athletic Association (NCAA) competitions. SS were recruited through direct contact in entry level science classes, fliers posted across the UAF campus, as well as social media. A simple Google Forms questionnaire was used to pre-screen SS for current and past exercise habits. SS reported not engaging in regular moderate physical activity (defined as "physical activity that takes moderate effort and makes you breathe somewhat harder than normal") for more than 20 minutes per bout, one bout per week, over the previous three months.

Participants completed a questionnaire that included health history, age, sex, and race (Table 1.1). There were no significant differences between TS and SS with regards to age or sex. Study participants were predominantly white with no significant differences in race between groups.

After removal of shoes, socks, and heavy clothing, anthropometric measurements were taken by a registered nurse (Table 1.2). Using a TANITA TBF-300A body composition analyzer (Tanita Corporation of America Inc., Arlington Hills, Illinois) set to the standard position for all participants, weight and percent body fat were measured.

Both groups completed the International Physical Activity Questionnaire (IPAQ) Short Form in the presence of a researcher. These data were cleaned and analyzed using IPAQ Guidelines for Data Processing and Analysis. Data from one TS and one SS participant were excluded based on this analysis. Metabolic Equivalent of Task (MET) scores in minutes/week were determined for each participant and classified into one of three physical activity categories: walking, moderate, or vigorous.

Vitamin D intake was gleaned from dietary data collected using the ASA24 system. Participants were instructed to record food and supplement (vitamin D) intake from one typical week day and one typical weekend day. Data were analyzed according to ASA24 protocol.

Blood draws were performed during the last week of November and the first week of December. Participants were instructed to fast for 12 hours prior to the blood draw. Consumption of water was permitted during the fast. Blood samples were collected in EDTA tubes, placed on ice, and centrifuged within two hours of the draw. Plasma was aliquoted and stored at -80° C for later analysis.

A commercially available ELISA kit (Enzo Life Sciences, Farmingdale, New York) was used to measure 25(OH)D according to the manufacturer's instructions. Briefly, 25(OH)D from plasma samples and alkaline phosphatase conjugated 25(OH) vitamin D_3 bind competitively to sheep monoclonal 25(OH)D antibody. The antibody then binds to donkey anti-sheep IgG coated on the interior surface of the plate wells. Excess material is washed out, a substrate is added causing remaining alkaline phosphatase conjugate to turn yellow, and plate absorbance is read at 405nm with an optical plate reader. Sample 25(OH)D concentrations were extrapolated from a standard curve.

Consensus has not been reached on what constitutes "optimal" levels of serum 25(OH)D nor have definitions of sufficiency, insufficiency, or deficiency been standardized. The Institute of Medicine (IOM) classification for serum 25(OH)D concentrations was employed for this study: ≥50 nM = sufficient, 30<50 nM = insufficient, and <30 nM = deficient. However, the Endocrine Society Guidelines state that a 25(OH)D level of 75 nM is required for sufficiency [28]. Öhlund et al [29] cite several reports for their use of the following scale: ≥75 nM = optimal, 50<75 nM = suboptimal, 37<50 nM = insufficient, and <37 nM = severely deficient. Though limited studies have reported optimal 25(OH)D levels for human athletic performance, it has been proposed that higher serum 25(OH)D levels (>100 nM) may improve athletic performance [30].

GraphPad Prism version 5.0d (GraphPad Software, Inc., La Jolla, California) was used for data analysis. A two-tailed, unpaired t-test was applied to evaluate differences between groups in regards to anthropometric data, IPAQ categorical comparisons, overall caloric intake, and plasma 25(OH)D concentration. A Mann-Whitney U test was used to assess physical activity levels and daily vitamin D intake for TS and SS. Male TS data were also compared to male SS.

Female TS data were compared to female SS. Differences were considered significant at $P \leq 0.05$. Data are presented as mean ± standard deviation unless indicated otherwise.

Results

Subject Characteristics

Table 1.1 outlines the demographics of TS and SS. There were no significant differences between TS (n=16) and SS (n=15) in age (TS=20.1±2.0, SS=21.5±2.1) or sex (male TS=8, female TS=8, male SS=8, female SS=7). All of the TS participants identified their ethnicity as white. Of the SS group, fourteen indicated their race as white, one indicated Alaskan Native (AN), and two reported as both AN and white.

Analysis of anthropometric data (Table 1.2) revealed significantly lower percent body fat and body mass index (BMI) in male TS (10.88±4.02, 21.84±1.69) as compared to male SS (19.84±6.15, 26.04±4.14). All members of the TS group had a BMI in the healthy range (18.6-25.0 kg/m^2) while 10 of the SS had a healthy BMI, three were classified overweight (25.1-29.9 kg/m^2) and two were classified obese (≥ 30 kg/m^2). Female TS did not show a significant difference in percent body fat or BMI compared to female SS. Furthermore, there were no significant differences in height, weight, or basal metabolic rate (BMR) between TS and SS for either sex.

Physical Activity

Walking, moderate, vigorous, and total MET scores in minutes per week were determined for each study participant. IPAQ analysis revealed one TS participant with moderate physical activity level while all other participants in the TS group sustained high physical activity levels. Conversely, SS participants demonstrated low physical activity levels except for

three with moderate and two with high levels. However, both MET-minutes/week (U=7.00) and categorical ranking of physical activity levels (U=17.5) were found to be significantly different between TS (n=15) and SS (n=14) groups. TS and SS groups demonstrated comparable walking MET scores (1134.10±578.49, 842.68±1166.34; Figure 1.1). Moderate, vigorous, and total METs were significantly greater in the TS group (1864.00±1085.57, 3952.00±2549.41, and 6910.10±2669.78) compared to the SS group (127.14±200.55, 257.14±778.91, and 1226.96±1450.22; Figure 1.1).

Vitamin D Intake

Total vitamin D intake for each study participant was analyzed as well as the source: food consumption or supplementation (Figure 1.2). Intake of vitamin D from food (Figure 1.2, A) was significantly greater in the TS group (316.623±160.79, U=37) than the SS group (113.184±110.13). Of importance, overall caloric intake was significantly higher for TS (3022±951.04) as compared to SS (2041±756.23). Male TS (348.00±176.3, U=8) consumed significantly more vitamin D from food compared to male SS (72.09±26.88) whereas the intake of vitamin D from food in female TS and SS was not significantly different (285.2±148.6, 160.10±150.40, U=13). It is noteworthy that all participants had vitamin D intake from food sources below the Recommended Dietary Allowance (RDA) of 600 IU per day.

Fourteen of 16 (88%) TS reported taking vitamin D supplements on one or both food recall days as compared to six of 15 (40%) SS. Vitamin D supplementation (Figure 1.2, B) was significantly greater for male TS (1044.00±1445.00, U=10.5) than male SS (146.60±414.70) and for sexes combined for TS (820.296±1127.87, U=67.5) than SS (431.533±1091.28). There was no significant difference in vitamin D supplementation between TS and SS females (596.90±723.6, 757.10±1532.00).

The total daily vitamin D intake (Figure 1.2, C) was significantly greater for TS (1136.92±1205.70, U=50) than SS (546.52±1089.93). Nine (56%) TS and three (20%) SS met or exceeded RDA for this age group of 600 IU per day. Total daily IU intake for male TS (1391.72±1512.33) was significantly greater than male SS (222.09±430.48) whereas there was no significant difference in total intake between female TS and SS (882.10±824.30, 917.3±1502.00).

Plasma 25(OH)D Levels

Fasting plasma levels of 25(OH)D (Figure 1.3) were significantly lower in the TS group (32.24±21.69) than in the SS group (51.92±24.88). TS males (23.91±11.97) demonstrated significantly lower levels of 25(OH)D as compared to SS males (56.93±23.14) whereas TS and SS females had comparable concentrations (40.56±26.57, 46.20±27.36). Furthermore, 44% of the TS participants were either insufficient (31%) or deficient (13%) in 25(OH)D while none of the SS were insufficient and 13% were deficient. The remainder of participants in both groups exhibited adequate levels.

Discussion

The purpose of this study was to evaluate physical activity, vitamin D intake, and fasting plasma 25(OH)D levels in trained student-athletes (TS) compared to sedentary students (SS) living in Fairbanks, Alaska, at 64° N. Sampling was conducted in November/December so that most, if not all, of vitamin D in the study population would need to be obtained from diet. Low sun angles at this time of year in conjunction with the half life of vitamin D would limit or eliminate conversion or storage of vitamin D from sunlight at 64°N. Physical activity was a lifestyle factor that was significantly different between the two groups. Most interestingly, TS

consumed a greater total dietary intake of vitamin D, yet had significantly lower plasma levels of 25(OH)D as compared to SS. Our hypothesis that TS are at greater risk for 25(OH)D insufficiency or deficiency as compared to sedentary counterparts is supported by these data.

Ergocalciferol (vitamin D_2) and vitamin D_3 are fat-soluble secosteroid compounds that are essential to human health. D_2 is synthesized by plants and fungi and may serve as a dietary source for humans [10]. D_3 is produced by conversion of 7-dehydrocholesterol in the skin via UV radiation. This transformation provides the most readily available source of D_3 for human metabolism [29]. Vitamins D_2 and D_3 are inactive molecules that undergo two hydroxylation reactions for activation. In a step-wise fashion, D_2 and D_3 are converted to 25(OH)D in the liver before forming calcitriol ($1,25(OH)_2D$) in the kidney. Concentrations of $1,25(OH)_2D$ in serum are tightly regulated by parathyroid hormone, calcium, and phosphate [31], with a circulating half-life of approximately 15 hours [32]. In contrast, 25(OH)D has a variable half-life of 15-45 days [33] making it a better indicator of vitamin D status and the standard measurement for research and clinical trials.

D_3 may also be ingested, though few unfortified foodstuffs provide sufficient concentrations to support adequate human health. Unfortified foods which are the exception include oily fish such as salmon, mackerel, herring, and cod liver oil [23]. Oily fish are thought to have been an important source of vitamin D for indigenous peoples of the circumpolar north. In fact, shifts away from traditional subsistence diets towards more westernized diets are likely lending to increasing prevalence of vitamin D deficiency and insufficiency in Alaska Natives [34]. Participants in this study were of predominantly European decent, did not live on a subsistence diet, nor did they consume higher than normal intake of oily fish.

Vitamin D insufficiency and deficiency has been identified as a worldwide health

problem [23]. In a meta-analysis of 394 studies with over 33,000 subjects worldwide, Hagenau et al [35] found mean 25(OH)D concentrations of 53.9±1.0 nM which is considered suboptimal or borderline insufficient. Furthermore, elite and sub-elite level athletes appear to be at higher risk of vitamin D deficiency than others [14]. Explanation as to why athletes may be at higher risk of deficiency include the utilization of $1,25(OH)_2D$ in muscle recovery [36], increased use of indoor training facilities [14], and increased use of sunblock and restrictive uniforms [28]. Moreover, it has been reported that exposure to winter sunlight at latitudes greater than 52° does not promote vitamin D_3 synthesis in human skin from October through March [24] and that those living at latitudes higher than 42° are at increased risk for seasonal insufficiencies or deficiencies in 25(OH)D [37].

Few studies, however, investigated vitamin D status in outdoor athletes living at high latitudes. Most recently a large study over four years evaluated vitamin D status according to sun exposure and oral supplementation in Polish athletes training outdoors. As expected, athletes experienced the most severe hypovitaminosis in winter months. Interestingly, increased winter sun exposure in athletes from northern climes – made possible by travelling to locations closer to the equator – improved vitamin D status more than oral supplementation [20]. Two studies that are more similar in scale and design to this one reported exacerbated hypovitaminosis in both soccer players training at 53°N [22] and rugby players training at 44°N [21] in the winter months. Hypovitaminosis was present, albeit to a lesser degree, in summer months indicating that higher muscle mass of athletes require larger amounts of vitamin D [21]. Hence, athletes living and training in the subarctic are likely to be at even greater risk than the general population as well as athletes living and training at lower latitudes.

In this study, TS consumed a greater quantity of vitamin D via food sources than SS

which may be a result of food choices and/or significantly greater daily caloric intake (kcal) (3022.67±951.04) compared to the SS group (2040±756.32). Additionally, supplemental vitamin D intake was greater in TS than SS in both numbers of individuals taking vitamin D as well as the amount taken. Despite the higher intake, TS demonstrated significantly lower levels of plasma 25(OH)D compared to SS. Furthermore, nearly half of TS had insufficient or deficient levels of 25(OH)D while none of the SS were insufficient and only two were deficient.

These data demonstrate that male TS have significantly lower 25(OH)D levels than male SS; females showed no difference between groups. We speculate that this discrepancy between sexes was a result of significant differences in body composition seen in the males but not the females. Research on body composition and vitamin D status indicate that both adiposity and reduced mean muscle mass contribute to vitamin D deficiency. For instance obesity has long been associated with low 25(OH)D status [6], despite previous animal studies that establish adipose tissue as the major storage site for D_3 [38]. Epidemiological studies have demonstrated lower vitamin D levels associated with higher BMI [39, 40]. Our data contrast these findings; however, any conclusion based on body fat would be difficult in the current study given that only five of the study subjects were classified as overweight or obese. Research by Ko et al found that men with a lower appendicular skeletal muscle mass index (ASMMI) score were more likely to be vitamin D deficient [41]. The authors conclude that a positive relationship between 25(OH)D and muscle mass in men may exist. Our results are also in conflict with these findings because we would expect our TS males to have higher muscle mass than their sedentary counterparts. The BMI of well-trained athletes is influenced primarily by their body fat content as they tend to display optimal muscle mass [42]. It is important to note however that ASMII and percent body

fat are not identical measurements. A larger sample size would provide clarity on the relationship between hypovitaminosis, muscle mass, and body fat composition.

Notwithstanding the small sample size, we showed significantly lower vitamin D levels in trained athletes living at high latitudes compared to their sedentary counterparts despite higher vitamin D intake. Our data suggest that this risk is more pronounced in males than in females. This study lends to the small, but growing body of evidence that athletes living at high latitudes are at an even greater risk of vitamin D insufficiency or deficiency than other individuals living at the same latitude. Further investigations are needed to determine if lower levels of plasma 25(OH)D in TS are due to an enhanced rate of skeletal muscle repair and/or other mechanisms unique to endurance athletics. These findings suggest that athletes living at high latitudes would benefit from regular vitamin D screening and oral supplementation.

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Table 1.1: Demographic data for trained athlete students and sedentary students living at 64°N.

Parameter	Trained	Sedentary
n	16	15
Age[M]	20.1 ± 2.0	21.5 ± 2.1
Gender		
Male	8	8
Female	8	7
Race		
White	16	14
Black	0	0
Alaskan Native	0	3[B]
Asian	0	0

Demographic data as reported on health history form. [M]Data reported as mean ± standard deviation. No significant differences between groups for age or gender. [B]Two participants of the sedentary group identified as both White and Alaskan Native ethnicity.

Table 1.2: Anthropometric data for trained athlete students and sedentary students living at 64°N.

Measurement	Trained Mean	Sedentary Mean[F]	P value
Height (cm)			
Male	179.0 ± 7.47	177.93 ± 6.73	0.7591
Female	166.2 ± 6.02	161.8 ± 9.78	0.3131
Weight (kg)			
Male	70.13 ± 9.24	82.96 ± 14.4	0.0512
Female	59.74 ± 7.06	61.42 ± 18.65	0.8126
Body Fat %			
Male*	10.88 ± 4.02	19.84 ± 6.15	0.0039
Female	23.45 ± 5.59	25.30 ± 11.06	0.6832
Body Mass Index (kg/m^2)			
Male*	21.84 ± 1.69	26.04 ± 4.14	0.0188
Female	21.65 ± 2.04	23.44 ± 6.47	0.4686
Combined*	21.74 ± 1.81	24.83 ± 5.32	0.0368
Basal Metabolic Rate (kcal/day)			
Male	1787 ± 153.4	1956 ± 218.0	0.0943
Female	1445 ± 77.27	1446 ± 190.7	0.9950

Data reported as means ± SD. *significantly different ($p < 0.05$). [F]Female participants in this group n=7, all other groups n=8.

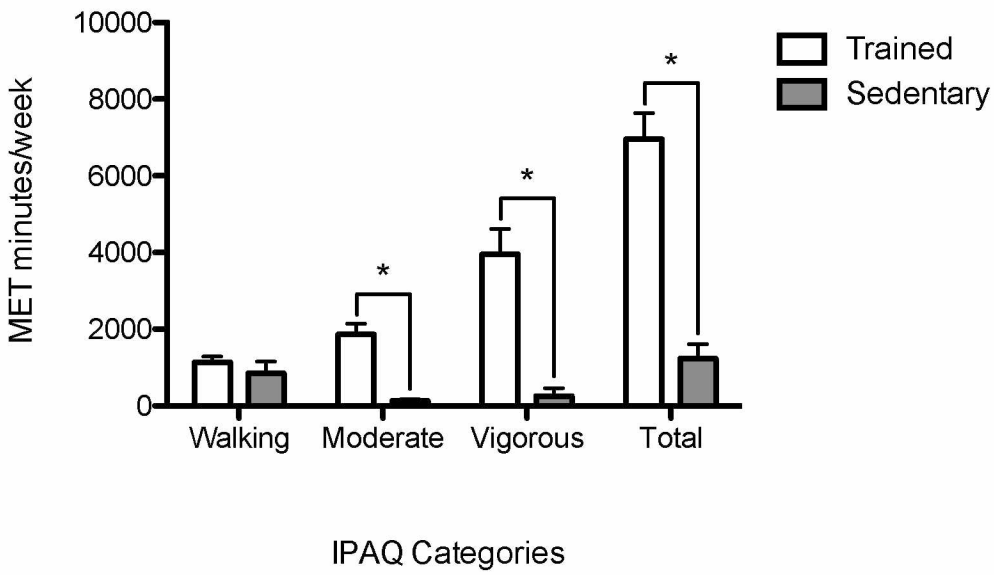
Figure Captions

Figure 1.1: Physical activity (walking, moderate, vigorous, and total) reported in MET-minutes/week for trained student athletes (open bars) and sedentary students (solid bars). Analysis was conducted according to International Physical Activity Questionnaire guidelines for data processing. Each bar represents the group mean ± SEM. *$p<0.05$.

Figure 1.2: Vitamin D (IU) from (A) food, (B) supplementation, and (C) total intake in trained student athletes (open bars) and sedentary students (solid bars). Data reported for males, females, and sexes combined. Data were obtained via the National Cancer Institute's Automated Self-Administered 24-hour Recall system. Each bar represents the group mean ± SEM. *$p<0.05$.

Figure 1.3: Fasting plasma 25(OH)D levels in trained student athletes (open bar) and sedentary students (solid bar). Data reported for males, females, and sexes combined. 25(OH)D levels were quantified by ELISA. Each bar represents the group mean ± SEM. *$p<0.05$.

Figure 1.1


10000
8000
6000
4000
2000
0
MET minutes/week
Walking
Moderate
Vigorous
Total
*
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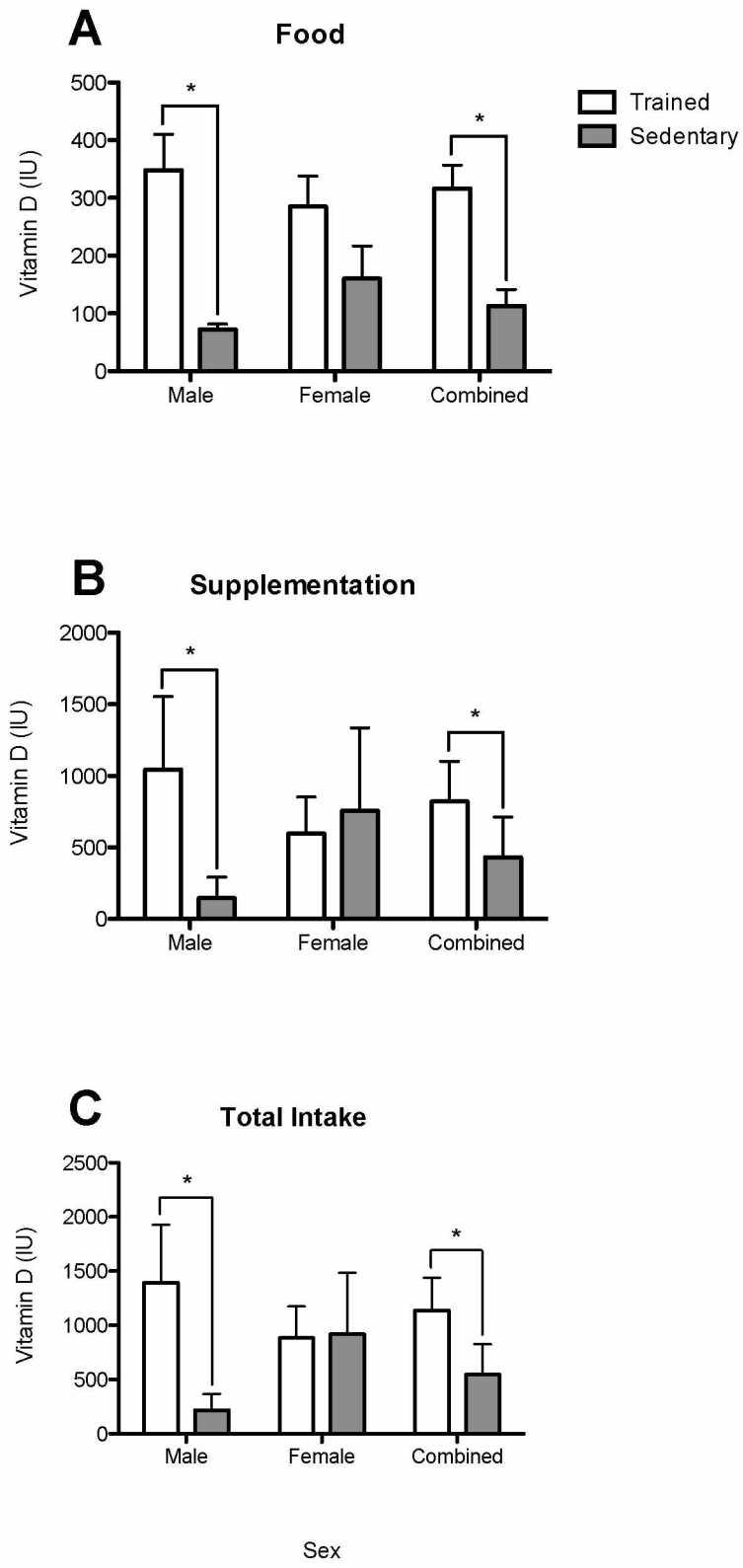
Trained
Sedentary
IPAQ Categories

Figure 1.2


A
Food
Trained
Sedentary
Vitamin D (IU)
500
400
300
200
100
0
*
*
Male
Female
Combined
B
Supplementation
Vitamin D (IU)
2000
1500
1000
500
0
*
*
Male
Female
Combined
C
Total Intake
Vitamin D (IU)
2500
2000
1500
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500
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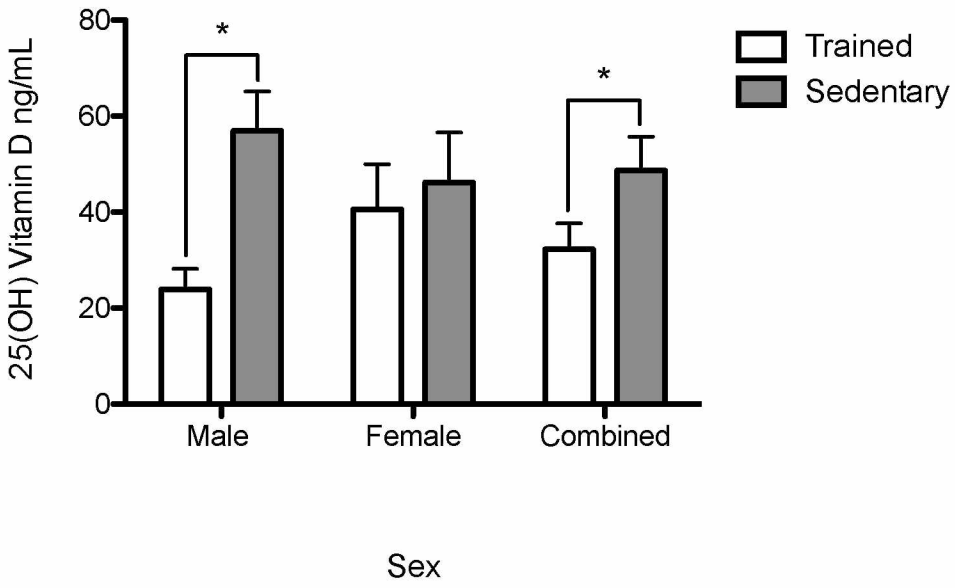
Female
Combined
Sex

Figure 1.3


80
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25(OH) Vitamin D ng/mL
*
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Sex
Trained
Sedentary

Chapter 2: Seasonal changes of vitamin D and cognitive function in collegiate cross country skiers at 64° north[1,2]

Abstract

PURPOSE: Vitamin D deficiency is well-documented in populations living above 37° N and more pronounced during winter months. Athletes are more susceptible to deficiency than non-athletes. Vitamin D has been linked to cognitive processes. Winter-sports athletes residing in the circumpolar North may exhibit vitamin D deficiency and depressed cognitive abilities during the competition season. This study aimed to determine if there are significant differences in vitamin D and cognitive function in winter athletes during mid-competition season versus post-season in the circumpolar North.

METHODS: Fifteen competitive cross country skiers residing at 64° N volunteered for this study. Blood samples were taken in early February ("mid-season") and late April ("post-season"). Subjects completed the Purdue Pegboard Test (PPT), an assessment of cognitive function, at the time of the blood draws. Plasma 25-hydroxyvitamin D [25(OH)D]

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[1] Scott P. Jerome[1], Kriya L. Dunlap[1,2], Hannah T. Short[3], Rebecca L. Gever[3], Lawrence K. Duffy[1,2], Andres D. Dajles[4], and Arleigh J. Reynolds[5]

[1]Department of Chemistry & Biochemistry, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[2]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[3]Department of Biology & Wildlife, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[4]Biomedical Learning & Student Training Program, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[5]Department of Veterinary Medicine, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[2] Prepared for submission, Medicine & Science in Sports & Exercise

concentration was measured as using an ELISA. Significance was determined by Wilcoxon and Mann-Whitney tests.

RESULTS: Subjects exhibited significantly lower ($p<0.05$) mean concentration of plasma 25(OH)D in post-season (8.45 nM ± 2.92 SEM) as compared to mid-season (14.81 ± 2.17). Mean PPT scores were significantly higher in post-season than mid-season (dominant hand, $p<0.05$; non-dominant hand, $p<0.01$; assembly, $p<0.001$; both hands $p<0.0001$) during post-season recovery.

CONCLUSION: Post-season 25(OH)D concentration was significantly lower than mid-season. PPT scores improved significantly in all four batteries in the post-season; 25(OH)D may not necessarily be linked to cognitive function measured with PPT. Mean 25(OH)D from both mid- and post-season fell below the Institute of Medicine's definition of "deficient" suggesting that subjects' improvement on PPT may have been attributable to another factor(s) such as reduced stress levels.

Introduction

Vitamin D insufficiency and deficiency are endemic worldwide [1, 2, 3]. Over the past 30 years vitamin D has been widely investigated with a sharp uptick in published work since 2003. One of the most studied and earliest understood roles of vitamin D is its regulation of mineral balance in bone metabolism. Researchers in the early 20th century began to understand that vitamin D supplementation could be used for the prevention and treatment of rickets in children and osteomalacia in adults [4, 5]. While this classical role of vitamin D is the most observable and best understood, vitamin D also has pleiotropic effects in a variety of human cell types [6]. Vitamin D insufficiency or deficiency has been linked with diabetes [6], cancer [1], thyroid dysfunction [7], low cardiac volume [8], impaired immune-system function [9], obesity [10], reduced cognitive function [11], metabolic syndrome, and cardiovascular disease [6]. While not conclusive, researchers have demonstrated that vitamin D sufficiency is positively correlated to proper muscle function [12, 13] and skeletal muscle repair and recovery [14]. Furthermore, muscle pain, weakness, and inadequate repair have been associated with vitamin D deficiency [14]. Clearly vitamin D's effects extend beyond bone health.

Though not uniformly accepted [15], recent studies suggest that some athletes do not maintain sufficient levels of 25-hydroxyvitamin D [25(OH)D] [16]. In a meta-analysis of 23 studies including 2,313 athletes, Farrokhyar et al. found that 56% displayed inadequate plasma vitamin D levels (<80 nM) [17]. While the Farrokhar study did find that inadequacy varied significantly with geographical location, few other studies have investigated this circumstance with relation to latitude [18]. For the North, the predominant hypothesis asserts that small sun angles in winter months at latitudes greater than 42° N result either in limited, severely limited, or insignificant conversion of 7-dehydrocholesterol by keratinocytes in the skin to vitamin D_3

[19, 20]. A cross-sectional study of 2,548 adults in Norway, revealed that 64% of participants were vitamin D deficient in winter (<50 nM) and 20% in summer. The researchers reported an overall rate of deficiency at 40% [2]. A greater risk of vitamin D deficiency exists in populations living at high latitudes as compared to populations living closer to the equator [21, 22, 23]. In a previous study our team found that trained cross country skiers living at 64° north had lower circulating vitamin D than their sedentary counterparts despite greater intake through diet and supplementation [24]. These studies, in addition to those proposing that athletes are at greater risk of deficiency than non-athletes, suggest that the overall risk for athletes living and training at high latitudes may be increased.

Vitamin D receptor (VDR) and the enzyme responsible for hydroxylating vitamin D to its active form, 1 alpha-hydroxylase, have been identified in human brain tissue [25]. A positive correlation between cognitive function and 25(OH)D concentration has been demonstrated in numerous studies. Vitamin D deficiency and insufficiency are directly correlated with performance on cognitive tests [26]. This was especially prevalent in finger tapping and hand movement tests similar to the Purdue Pegboard Test [27].

This study had two primary goals: first, to determine if differences in vitamin D levels and cognitive function exist in trained endurance athletes between the middle of the competition season and during the post-season recovery phase; and second, to compare cognitive function test scores with the 25(OH)D levels of individual participants who reported a drop in their athletic performance during the season.

We hypothesize that 25(OH)D levels will be significantly higher in trained endurance athletes after one month of recovery from the competition season than during mid-season. Also, subjects will score significantly lower on cognitive function tests during the mid-season than

during recovery, and that subjects who experience a decrease in athletic performance during the season will have lower mean cognitive function scores.

Methods

The University of Alaska Fairbanks (UAF) Institutional Review Board approved this study (#838437-5) prior to data collection. Also prior to data collection, written consent of all participants was obtained after explanation of the study, including risks and benefits. A presentation by the researchers was given to members of the UAF cross country ski team; attendance was optional. The presentation outlined current research questions, the specific purpose of this project, opportunities to volunteer for the project as study participants, potential risks of participation, and anticipated benefits of participation. Fifteen members of the ski team volunteered to participate (Table 2.1). A power analysis of exercise science studies utilizing human athletes was completed prior to participant recruitment. Our analysis suggested that a group of 12 would be sufficient for determining significant differences in vitamin D parameters. All participants were preparing for, and competing in, National Collegiate Athletic Association (NCAA) cross country skiing competitions. Participants completed the International Physical Activity Questionnaire (IPAQ) to assess overall activity level through metabolic equivalent task (MET) minutes; all participants were rated as meeting "health enhancing physical activity" according to IPAQ guidelines.

Participants completed questionnaires, had blood drawn, and completed a simple test of cognitive function at two different times during their athletic calendar: early February ("mid-season") and late April ("post-season recovery"). The February sampling occurred at the approximate midpoint of the competitive ski season. The April sampling took place after

approximately one month of recovery following the conclusion of the season. Participants were asked to fast for 8-10 hours prior to each testing date/time and to refrain from exhaustive physical activity during that time period. Consumption of water was allowed during the fasts. Questionnaires were administered by a trained researcher prior to anthropometric measurements and blood draws. The first questionnaire surveyed the participants regarding athletic performance, unexplained drops in athletic performance, activity levels, and vitamin D supplementation. The IPAQ Short Form surveyed the participants in greater depth about physical activity levels.

Following the completion of questionnaires and prior to blood draws a trained medical student measured height, weight, and body composition using a TANITA TBF-300A body composition analyzer (Tanita Corporation of America Inc., Arlington Hills, Illinois) set to the standard position for all participants. Blood draws were performed by a registered nurse trained in phlebotomy. Blood samples were collected in three tubes: 3.5 mL serum separation, 4 mL EDTA, and 10 mL EDTA. Through the UAF Student Health and Counseling Center the 3.5 and 4 mL tubes were sent to an outside lab for processing (Quest Diagnostics, Secaucus, New Jersey). The 10 mL tubes were placed on ice and centrifuged within 30 minutes of the draw. Plasma was aliquoted immediately, flash frozen, and stored at -80° C for later analysis.

Following the blood draw, participants performed the Purdue Pegboard Test (Lafayette Instrument, Lafayette, Indiana) under the supervision of a trained researcher. The Purdue Pegboard Test (PPT) is a simple test of neuropsychological skills and cognitive function [28]. Participants completed four separate timed tasks. The first three -- dominant hand, non-dominant hand, and both hands simultaneously – required the participant to place small, metal pegs in holes. The final test required the assembly of a small pieces of hardware using metal

pegs, washers and collars with both hands. Each task was performed three times with the mean of each serving as the final score.

To measure 25(OH)D, a competitive, enzyme-linked, immunosorbent assay (ELISA) kit (Enzo Life Sciences, Farmingdale, New York) was used according to the manufacturer's instructions. Plasma 25(OH)D from samples and alkaline phosphatase conjugated 25(OH) vitamin D_3 compete to bind to sheep monoclonal 25(OH)D antibody. The bound antibody then binds to donkey anti-sheep IgG that is coated on the interior surface of the plate wells. Excess material is washed from each well, a substrate is added causing remaining alkaline phosphatase conjugate to turn yellow, and well-color is read at 405nm with an optical plate reader (Synergy HT, BioTek Instruments, Winooski, Vermont). Sample 25(OH)D concentrations are then extrapolated from a standard curve.

Statistical analysis was performed with Prism (versions 5, 6 &7, Graphpad Software, Inc.). D'Agostino & Pearson omnibus test was used to determine normality of data sets. If normal distribution was found, data were compared with a paired t-test. If data were not normally distributed it was compared with the Wilcoxon matched-pairs signed rank test. Unpaired data sets were analyzed with the Mann-Whitney test. Correlations were determined with Spearman's rank correlation coefficient. Significant differences are reported at $P \leq 0.05$. All error is reported as standard error of the mean (± SEM).

Results

Participants reported significantly lower weekly mean MET minutes in post-season recovery (3185 ± 446.1) as compared to mid-season (5336 ± 427.4). There were no significant differences in percent body fat for all participants, males, or females between mid-season and post-season

recovery (Table 2.1). Mean plasma 25(OH)D concentration was significantly lower (P = 0.0084) in post-season recovery (8.45 ± 2.92 nM) than mid-season (14.82 ± 2.17 nM) (Figure 2.1). When analyzed according to sex, we found that the male group displayed significantly lower plasma 25(OH)D concentration in post-season recovery (4.32 ± 1.30 nM) than mid-season (15.48 ± 3.08 nM) while the female group revealed no significant difference between post-season recovery (13.18 ± 5.79 nM) and mid-season (14.08 ± 3.27 nM) (Table 2.2). There was no significant difference in mean 25(OH)D between males and females in the mid-season nor in post-season recovery.

Participants scored significantly higher in all four PPT batteries in post-season recovery than mid-season (Figure 2.2). We found a significant, positive correlation between plasma 25(OH)D concentration and the PPT assembly battery (P = 0.049) in post-season recovery. However, we found no other significant correlations between 25(OH)D and PPT batteries in either post-season recovery or mid-season. There was no correlation between percent body fat and 25(OH)D.

Six participants reported experiencing at least one drop in athletic performance lasting three weeks or longer during the ski season. Mean 25(OH)D for these six (6D) was significantly lower post-season (4.20 ± 1.81 nM) than mid-season (14.05 ± 3.85). Mean 25(OH)D concentrations of the nine participants (9ND) who reported no drop(s) in performance lasting three weeks or longer at any time during the season displayed no significant difference between post-season (11.28 ± 4.58) and mid-season (15.34 ± 2.50) (Figure 2.3).

Among 6D and 9ND independently, we investigated correlations between plasma 25(OH)D concentration and PPT. Of the 16 possible combinations, only one displayed a

significant correlation. In the post-season condition, 6D displayed a positive correlation between plasma 25(OH)D concentration and assembly battery performance.

For the total group, supplementation taken during the season was generally stopped in the post-season recovery. Reported weekly mean vitamin D intake from oral supplementation was significantly lower in post-season recovery (2,333 ± 1876 UIs) versus mid-season (11,327 ± 3688). Three of the 15 participants reported taking a vitamin D supplement during post-season recovery; 11 reported taking a supplement during the mid-season. Of 6D, two reported supplementation in post-season recovery and five in mid-season. In the 9ND group, one reported supplementation in post-season recovery and six in mid-season.

Discussion

There is little consensus within the exercise science community regarding what constitutes "optimal" levels of 25(OH)D for athletic performance [29, 30]. At the lower end of the scale, what defines a "sufficient" level of vitamin D for basic human health also remains controversial [31, 32]. The Endocrine Society guidelines state that plasma 25(OH)D concentration of 75 nM is required for sufficiency [33]. Öhlund, Silfverdal, Hernell, & Lind cite several reports for their use of the following scale: ≥75 nM = optimal, 50>75 nM = suboptimal, 37>50 nM = insufficient, and ≤37 nM = severely deficient [29]. For the purpose of this study we used a conservative scale from the Institute of Medicine (IOM) to classify plasma 25(OH)D concentrations: ≥50 nM = sufficient, 30>50 nM = insufficient, and ≤30 nM = deficient. The mean of all participants displayed vitamin D deficiency in both the mid-season (13.94 ± 2.41 nM) and post-season recovery (8.45 ± 2.92 nM). As reported, the post-season recovery mean value was significantly lower than mid-season. This data refute our hypothesis that mean

25(OH)D level would be significantly higher in post-season recovery. This may be attributable to the fact that only four of the 11 participants who reported taking a vitamin D supplement in the mid-season continued to take a supplement in post-season recovery. Weekly mean vitamin D intake from supplements, inclusive of all participants, dropped from 11,327 IUs to 2,333. Both the IOM and the United States Department of Agriculture recommend a daily allowance of 600 IUs (4,200/wk). The participants' post-season recovery mean supplementation falls far below these recommendations. Though we did not assess participants' vitamin D intake through diet, our data add to the evidence that people living in the far North are unable to produce virtually any vitamin D in the skin via UV radiation in early spring due to low sun angles [34]. Therefore, vitamin D supplementation and/or a diet plentiful with foods rich in vitamin D – fortified and/or naturally occurring – is required to achieve recommended plasma 25(OH)D concentration.

One proposed pleiotropic effect of vitamin D is muscle trophicity. Specific populations experience increased demand of vitamin D from muscle damage, atrophy, or loss, such as athletes or the elderly. They may require higher production and/or intake of vitamin D in order to maintain or increase muscle mass [35]. Our data suggest that participants on average were vitamin D deficient within a period of high exercise exertion (mid-season) as well as during a recovery phase (post-season). Muscle function nor lean body mass was tested for. A follow-up study encompassing an entire training/competition cycle (year) with quarterly sampling and testing to include muscle force production, exercise time to exhaustion, and total body composition will determine if a correlation exists between vitamin D and muscle function and mass in northern athletes.

The significant decrease from mid-season to post-season recovery in plasma 25(OH)D concentration was at least partially driven by the male participants. The female participants

showed no significant difference in 25(OH)D between mid-season and post-season recovery, while the males displayed a significant reduction of over 66%. Despite the significant difference in mean 25(OH)D in males from mid-season to post-season recovery, there were no significant differences between male and female 25(OH)D in either the mid-season or post-season conditions. In the mid-season, 11 (5 = M, 6 = F) of 15 (8 = M, 7 = F) participants reported taking a vitamin D supplement; in post-season recovery only three participants (1 = M, 2 = F) reported doing so. Therefore, it is unlikely that supplementation explains the difference in plasma 25(OH)D between the sexes. Further research with a more robust sample size is warranted to investigate if correlations exist between sex, adiposity, and vitamin D status among northern athletes. In the lower populated Arctic and sub-Arctic, cross-regional groups would be needed to generate a sufficient number of subjects. However, this would introduce more heterogeneity in environmental factors.

The Purdue Pegboard Test was employed in order to measure cognitive function. Vitamin D insufficiency and deficiency have been linked to decreased cognitive function, though primarily in the elderly [27]. Our findings indicate that while plasma 25(OH)D concentration dropped significantly from mid-season to post-season recovery, PPT scores rose significantly from mid-season to post-season recovery. This contradicts our hypothesis, though the scope is limited. The method used, specifically the dates of testing, did not permit testing participants when plasma 25(OH)D concentrations would likely be the highest (late summer). The current study revealed that all participants were vitamin D deficient at the time of both PPT tests. Interestingly, we found significant, positive correlations between 25(OH)D and the assembly battery for all participants in post-season recovery and for 6D in the post-season recovery. The assembly battery, requiring the use of both hands to build a small component from four small

metal pieces, is the most complex battery of the PPT. Though far from conclusive, these correlations do support a hypothesis that vitamin D levels and cognitive function in athletes are positively linked. The overall rise in PPT scores may be explained by another factor altogether. Total stress and cumulative fatigue experienced by the participants may have been substantially lower in April than in February. The competition season is characterized by multiple intercollegiate ski competitions, multiple trans-continental flights, darkness, and extremely cold temperatures. The alleviation of some stressors – conclusion of competition, end of athletic travel, increased daylight, moderating temperatures – may explain higher PPT scores in post-season recovery [36, 37]. Repeating the PPT and 25(OH)D screening with polar region athletes and controls monthly during the year may help to reveal if a link between mental processes and vitamin D exists within this population.

Improvement in exercise performance is based on the simple principle of overload and recovery [38]. If a body is stressed with a workload that is greater than what is normally experienced and then is allowed to recover, a "supercompensation" effect occurs. The body reacts to the stress of the higher workload by becoming stronger and a new level of fitness is thus attained [38]. This normal overload and recovery process is termed "functional overreaching" (FO). However, when the workload is set too high or, more likely, when the recovery period is inadequate, athletic performance can stagnate, a condition known as "non-functional overreaching" (NFO). Functional overreaching and NFO exist on a spectrum with a third possible outcome called "overtraining" (OT). Overtraining is characterized primarily by an unexplainable, sustained drop in athletic performance despite seemingly normal training cycles [39]. If the workload in OT is too great and/or the recovery is not sufficient and performance drops. Overtraining syndrome (OTS) is a maladaptation to training causing a long term decline

in athletic performance. Recovery may require weeks, months, or years, while some never completely recover or reach pre-OTS performance [40]. OTS has several symptoms, but the drop in athletic performance is often the most pronounced. It is proposed that athletic training is not the only contributing factor to OTS [39]. While there is no test for OTS, it can be diagnosed by a healthcare professional following a thorough review of the athlete's training/competition history and diet history, and by ruling out all other possible causes for the decline in performance [39]. Other causes for decreasing performance, which may eliminate the OTS diagnosis, include, but are not limited to, bacterial infection, viral infection, anemia, and negative caloric balance [40]. An athlete may still be diagnosed with OTS if any of the aforementioned conditions occurred as a result of OTS. While subjective, one possible marker of NFO or OT is an unexplainable drop in athletic performance lasting three weeks or longer [40]. Our study identified six individuals who reported at least one drop in performance lasting at least three weeks during the competition season.

There were no significant differences in mean 25(OH)D concentrations between 6D and 9ND in the post-season recovery nor mid-season conditions. When examining 6D independently we found mean 25(OH)D significantly lower in post-season recovery than mid-season. This decline in vitamin D was not mirrored in 9ND (Figure 2.3). Mean plasma 25(OH)D concentrations for 9ND showed no significant difference between mid-season and post-season recovery. We found that without 6D there would be no significant difference in mean plasma 25(OH)D concentration from mid-season to post-season recovery indicating that vitamin D may play a role in athletic performance. In addition to the influence of male participants, it is clear that all who reported a drop in athletic performance helped to drive mean 25(OH)D

concentration downward in the post-season condition. The 6D group is split evenly by sex, three female and three male.

This study examined plasma 25(OH)D concentrations, PPT scores, and athletic performance during the mid-season and post-season recovery in trained cross country skiers living in Fairbanks, Alaska, at 64° N. It revealed that study participants were vitamin D deficient in both testing conditions and vitamin D was significantly lower in the post-season recovery. Male participants and all who reported a drop in performance were largely responsible for the decline in plasma 25(OH)D concentration. A reduction in vitamin D supplementation may account for part of the change in plasma 25(OH)D concentration. Due to low sun angles, vitamin D cannot be synthesized in the skin via UV radiation from October through March at 64° N [34]. Scores on the PPT were significantly higher in post-season recovery. A reduction in athletic and environmental stressors and fatigue may account for part of the change in PPT scores. We discovered positive correlations between the assembly battery of the PPT and mean plasma 25(OH)D concentrations in the post-season condition for the entire group as well as those who reported a drop in athletic performance. Athletes who reported a drop in performance showed significantly lower mean plasma 25(OH)D concentration in post-season recovery. Our results suggest that athletes are important research subjects for understanding the relationship between plasma 25(OH)D concentration and human health in stressful environments like the circumpolar North.

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Conflicts of Interest

The authors have no conflicts of interest to report. The lead author had coached some of the volunteer participants a few years prior to the commencement of this study. The lead author was not coaching at the time of participant recruitment nor during any part of the study. In addition, the lead author was not involved in the administration of the cognitive function tests. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Table 2.1

	N	Age	Body fat (%)		Total MET† (min/wk)	
			Mid-season	Post-season	Mid-season	Post-season
Total	15	20.4 ± 1.64	NA	NA	5335 ± 427	3185** ± 446
Male	8	20.6 ± 1.92	12.0 ± 0.387	11.9 ± 0.573	4903 ± 449	3447 ± 735
Female	7	20.1 ± 1.35	29.4 ± 2.22	28.7 ± 2.29	5830 ± 754	2886* ± 495

Age, percent body fat, and MET minutes/week for all participants, males, and females. Mean values ± SEM. †Metabolic Equivalent Task. **$p<0.01$ *$p<0.05$

Table 2.2: Vitamin D concentration

	Mid-season	Post-season
Total	14.81 ± 2.17	8.45 ± 2.92 *
Male	15.48 ± 2.25	4.31 ± 0.95 *
Female	14.08 ± 3.27	13.18 ± 3.95

Plasma 25(OH)D concentration (nM) of the entire group (N = 15), males (8), and females (7) in the mid-season and post-season recovery. Mean values ± SEM. $p<0.05$.

Figure Captions

Figure 2.1. Mean plasma 25(OH)D concentration of 15 trained cross country skiers in the middle of the competition season ("mid-season") and following one-month recovery (post-season").

Figure 2.2. Purdue Pegboard Test results by individual battery during mid-season and post-season recovery, reported with mean values ± SEM. All values were significantly higher during post-season recovery. A. Dominant 17.04 ± 0.4689, 18.00 ± 0.3381; B. non-dominant 15.98 ± 0.4173, 16.89 ± 0.2889; C. both hands 12.98 ± 0.2591, 14.02 ± 0.2882; D. assembly 41.69 ± 1.128, 46.64 ± 0.8875.

Figure 2.3. Mean plasma 25(OH)D concentration during mid-season and post-season recovery of: A. athletes reporting at least one drop in performance lasting three weeks or longer during the competitive season; and B. athletes reporting no drop(s) in performance lasting three weeks or longer during the competitive season.

Figure 2.1

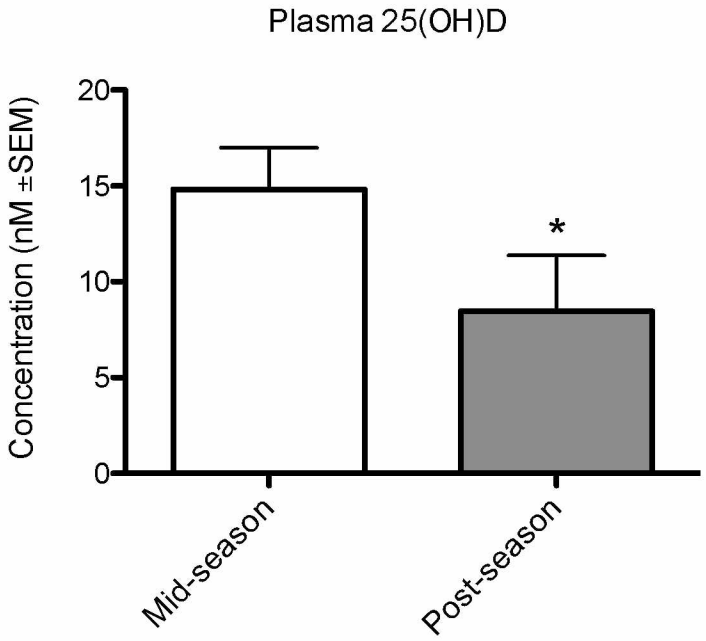

Plasma 25(OH)D
Concentration (nM ±SEM)
20
15
10
5
0
*
Mid-season
Post-season

Figure 2.2

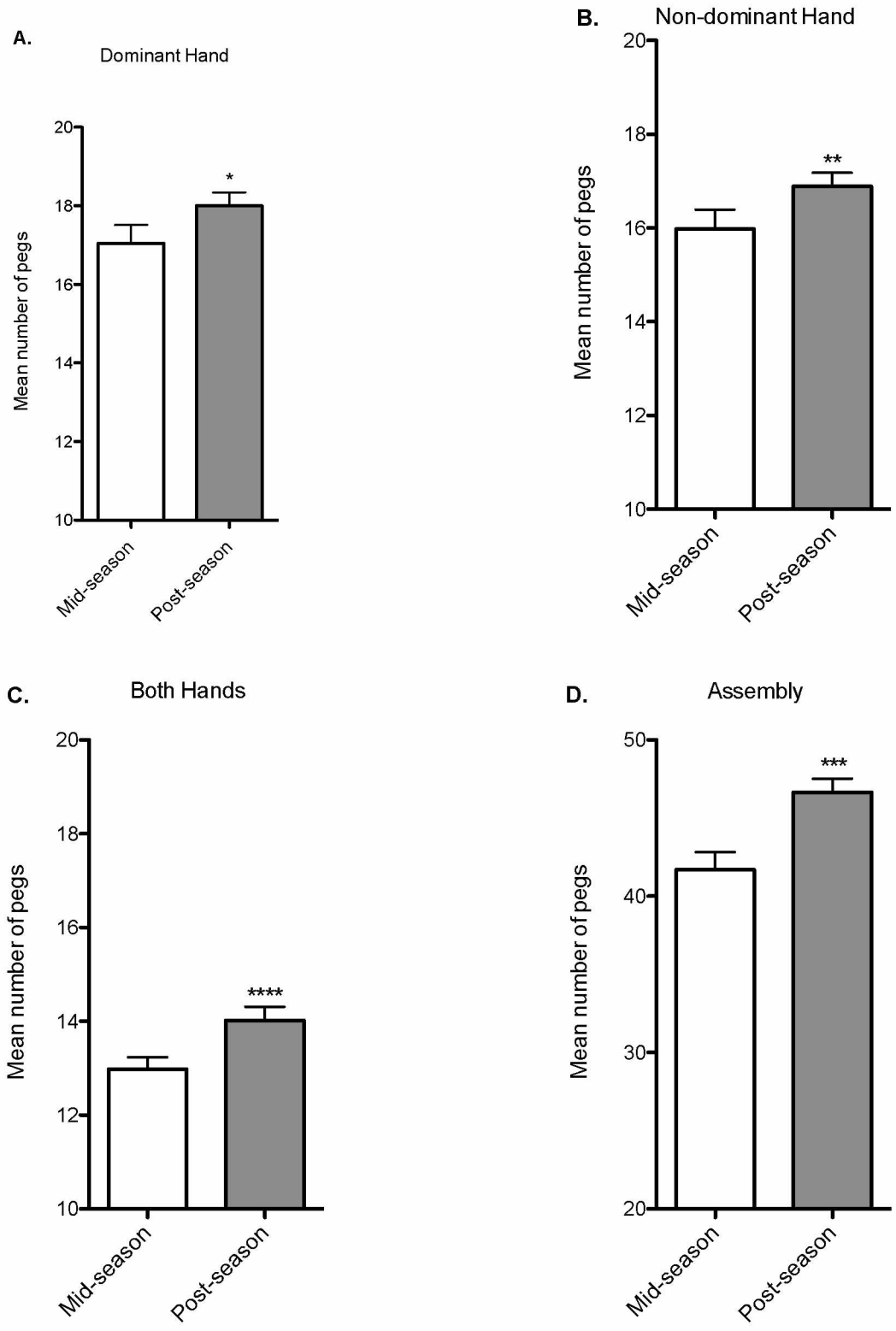
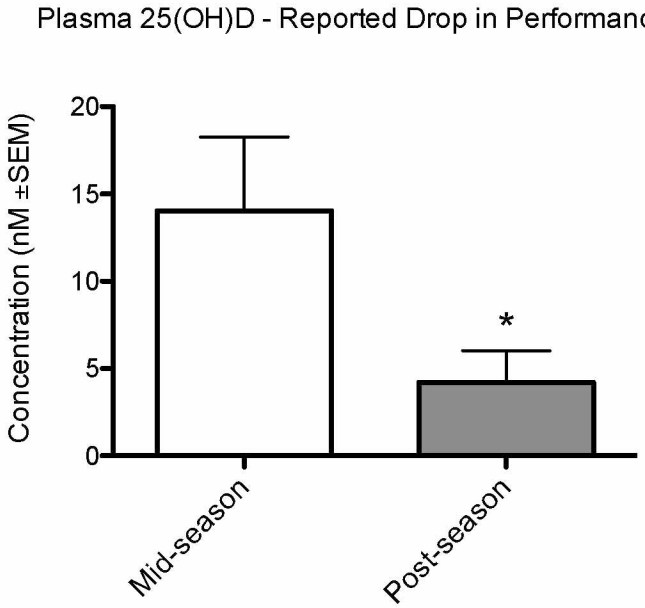
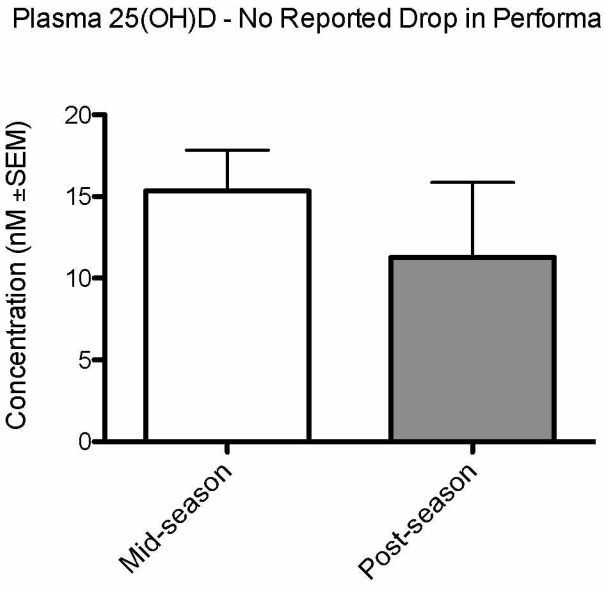

A.
Dominant Hand
Mean number of pegs
20
18
16
14
12
10
*
Mid-season
Post-season
B.
Non-dominant Hand
Mean number of pegs
20
18
16
14
12
10
**
Mid-season
Post-season
C.
Both Hands
Mean number of pegs
20
18
16
14
12
10
****
Mid-season
Post-season
D.
Assembly
Mean number of pegs
50
40
30
20
***
Mid-season
Post-season

Figure 2.3

A.


Plasma 25(OH)D - Reported Drop in Performance
Concentration (nM ±SEM)
20
15
10
5
0
*
Mid-season
Post-season

B.


Plasma 25(OH)D - No Reported Drop in Performance
Concentration (nM ±SEM)
20
15
10
5
0
Mid-season
Post-season

Chapter 3: Oxidative stress in collegiate cross country skiers in mid- and post-seasons[1,2]

Abstract

Purpose: The oxidative stress (OS) hypothesis of overtraining syndrome argues that increased production of free radicals through exercise cause muscle fatigue and damage resulting in lower athletic performance. Several studies have investigated OS immediately before and after exercise bouts in a training macrocycle. Out study aimed to compare OS of endurance athletes between a competition macrocycle and the immediate post-season recovery macrocycle. In addition, we aimed to identify athletes who experienced an unexplainable drop in athletic performance during the competition season in order to compare their OS to those who experienced no drop in performance.

Methods: Fifteen members of the University of Alaska Fairbanks cross country ski team volunteered for this study. Blood samples were taken in early February ("mid-season") and late April ("post-season"). Participants completed questionnaires regarding physical activity and athletic performance at the time of the blood draws. Plasma was analyzed for 4-hydroxynonenal

[1] Scott P. Jerome[1], Kriya L. Dunlap[1,2], Ava M. Parrish[1], Matthew L. Lowman[3], Emily M. Shipman[3], Lawrence K. Duffy[1,2], and Arleigh J. Reynolds[5]

[1]Department of Chemistry & Biochemistry, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[2]Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[3]Department of Biology & Wildlife, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[5]Department of Veterinary Medicine, University of Alaska Fairbanks, Fairbanks, AK, 99775, USA

[2] Prepared for submission, Medicine & Science in Sports & Exercise

(HNE), nitrotyrosine, nitric oxide (NOX), and superoxide dismutase (SOD). Significance was determined by Wilcoxon and Mann-Whitney tests.

Results: Participants displayed significantly higher ($p<0.05$) SOD activity in the post-season (0.02065 U/mL ± 0.006477 SEM) as compared to mid-season (0.04459 U/mL ± 0.005860). Six athletes reported an unexplainable drop in performance (6D). In the post-season, HNE concentration was significantly higher ($p<0.05$) for 6D (197.5 μg/mL ± 22.79) than for participants who did not report such a drop (69.80 μg/mL ± 33.59). 6D SOD activity was significantly higher in post-season (0.05048 U/mL ± 0.004688) than mid-season (0.01241 U/mL ± 0.006469).

Conclusion: Signs of oxidative stress and mitigation during the post-season recovery macrocycle were higher in athletes who reported experiencing a drop in athletic performance during the competition season macrocycle.

Introduction

Of all the diseases, injuries, and illnesses which can negatively affect performance of elite (world class) and sub-elite (collegiate, national, and regional level) endurance athletes, few are more frustrating for athletes, coaches, and physicians than overtraining syndrome (OTS). Characterized by an unexplainable drop in athletic performance, OTS symptoms can also include general malaise, lethargy, disrupted cognitive function, mood swings, depression, and sleep disorders [1]. Meeusen et al. describe OTS as a "prolonged maladaptation" of biological, neurochemical, and hormonal regulation systems [2]. The diagnosis of OTS may result when no other explanation for an athlete's prolonged drop in performance can be identified [1]. A recent search of the PubMed database produced 150 references for "overtraining syndrome", 73 for "overtraining syndrome diagnosis", and only two for "overtraining syndrome prediction". A 2012 Joint Consensus Statement on OTS from the European College of Sport Science and the American College of Sports Medicine identified no convincing evidence as to the causes of the condition [2]. To date, most published studies regarding OTS have focused on prevention, symptoms, diagnoses, and recovery [1, 2, 3, 4, 5, 6, 7]. A gap in the literature exists with regards to prediction. The basic question, "Who is most susceptible to developing OTS?" remains unanswered.

Several hypotheses exist regarding the etiology of OTS, ranging from chronic glycogen depletion to inflammatory cytokine release [1]. The oxidative stress hypothesis asserts that when oxidative stress becomes pathologic inflammation, muscle fatigue and soreness will result in reduced athletic performance [1]. Tanskanen, Atalay, & Uusitalo found indicators of higher oxidative stress in overtrained athletes than in controls both at rest and during exhaustive exercise, suggesting that oxidative stress plays a role in the pathophysiology of OTS [8]. A

study featuring a periodized training protocol for human participants culminated with an overtraining (OT) block resulting in significant increases in three oxidative stress markers (OSMs): isoprostanes, thiobarbituric acid-reactive substances, and protein carbonyls [9]. While studies have revealed a link between OSMs and OTS following acute exercise [8, 9], few if any have examined the relationship between oxidative stress and overtraining over a training or competition macro cycle.

Functional overreaching (FO) is defined as the normal overload, recovery, and super-compensation cycle required for athletic improvement. Nonfunctional overreaching (NFO) shares an overload feature with FO, but an ineffectual recovery period leads to stagnant performance. OT shares an overload period and ineffectual recovery with NFO. However, instead of a performance plateau, performance drops with OT [2]. These three outcomes to athletic training – FO, NFO, and OT – are not completely isolated responses but exist together on a stress/response continuum. This study examines the response to a macro training cycle by examining athletes' abilities to adapt to stress and reach homeostasis. While it is unclear if any of our participants were overtrained, six of the 15 reported at least one unexplained drop in athletic performance during the competitive season lasting three weeks or longer. Though not conclusive in and of itself, a lasting drop in athletic performance is one diagnostic index for OTS [1]. Our study examined differences in oxidative stress/status markers in athletes who reported at least one unexplained drop in performance during a competition season and those who did not. We sampled participants at two different times of the year: 1) in the middle of the competitive season; and 2) following one month of recovery in the post-season. We hypothesized that those who reported a drop in performance would display significantly greater oxidative stress than those who did not. Furthermore, we hypothesized that those who experienced no drop in

performance would show lower oxidative stress in the post-season condition because oxidative stress is associated with muscle fatigue [1]. Analyzing resting OSM values from mid-season and post-season recovery is a comparison few studies have undertaken. These data will add to the body of knowledge of OSMs during periods of exercise and recovery.

Methods

The study protocol was approved by the Institutional Review Board of the University of Alaska Fairbanks (UAF) (#838437-3). Following a thorough description of the study, including risks and benefits, 15 members of the UAF intercollegiate cross country ski team gave their informed consent to participate prior to data collection. The skiers were training for, and competing in, National Collegiate Athletic Association (NCAA) Division-I regional and national championship events. The group consisted of eight males and seven females (Table 3.1). All participants had been training for ten to twenty hours per week for no fewer than three months prior to sample collection. The first collection took place in early February, the middle of the competition season (mid-season). The second collection took place in late April, approximately four weeks following the conclusion of the competition season (post-season recovery). All samples were collected at least 15 hours post-exercise.

Participants completed the International Physical Activity Questionnaire (IPAQ) Short Form with instructions from a researcher at both data collection dates. Data from the questionnaires were cleaned and analyzed according to the IPAQ Guidelines for Data Processing and Analysis. A questionnaire to determine hours of athletic training and sport performance history was also administered at mid-season and post-season sampling.

Participants fasted for 12 hours prior to each blood draw, mid- and post-season, with nothing consumed except water. Blood was obtained via venipuncture by a registered nurse trained in phlebotomy. 17.5 mL of blood was drawn per participant into three tubes: 4 mL, 3.5 mL, and 10 mL. The 4 mL and 3.5 mL EDTA tubes were sent to an outside lab (Quest Diagnostics, Madison, NJ) for analysis while samples collected in the 10 mL EDTA tubes were centrifuged, aliquoted into 0.5 mL tubes, and flash-frozen within 30 minutes of sample collection. The resulting serum was stored at -80° C for future analyses.

Plasma samples were analyzed for superoxide dismutase (SOD) (Cayman Chemical, Ann Arbor, MI), nitrotyrosine†, 4-hydroxynonenal† (HNE), and nitric oxide† (NOX) (via nitrite and nitrate) (†Cell Biolabs, San Diego, CA) according to manufacturer's instructions.

A power analysis prior to study commencement indicated that a minimum of 12 participants would be necessary for validity. Statistical analysis was performed with Graphpad Prism (version 5, Graphpad Software, Inc.). Normality of data sets was determined using the D'Agostino & Pearson omnibus test. If normal distribution for a particular set was found, data were compared with a paired t-test. If data were not normally distributed, sets were compared with the Wilcoxon matched-pairs signed rank test. Unpaired data were analyzed with the Mann-Whitney test. Outliers were identified with the extreme studentized deviate method (Grubbs' test). Significant differences are reported at $P \leq 0.05$. All error is reported as standard error of the mean (± SEM).

We did not have adequate sample volume from two participants in the mid-season condition for the nitrotyrosine assay. The matched samples from the post-season recovery condition were subsequently not used for data analysis. One data point for SOD activity in the

mid-season condition was found to be an outlier. Neither this outlier nor its corresponding value from the post-season recovery condition were used in SOD analyses.

Results

The IPAQ data indicate that the participants were significantly more active in mid-season than during post-season recovery (Table 3.1). Six reported experiencing at least one period of unexplainable, reduced athletic performance lasting at least three consecutive weeks during the season. The remaining nine reported no such drop in performance.

No significant difference in mean HNE concentration between mid-season (95.77 μg/mL ± 26.68) and post-season recovery (120.9 ± 27.22) was detected. Among the six participants who reported a drop in performance (6D), there was no significant difference between mid-season (143.1 ± 36.12) and post-season recovery (197.5 ± 22.79). There was no significant difference in concentrations between mid-season (64.19 ± 34.94) and post-season recovery (69.80 ± 33.59) for the nine participants who did not report a drop in performance (9ND). In the mid-season condition, there was no significant difference between 6D (127.1 ± 43.09) and 9ND (74.91 ± 34.15). In the post-season recovery condition, HNE concentration for 6D (197.5 ± 22.79) was significantly higher than 9ND (69.80 ± 33.59) (Figure 3.1).

Our analysis of NOX through nitrite (NO_2^-) and nitrate (NO_3^-) concentration revealed no significant difference in mean values between mid-season (21.35 μM ± 3.331) and post-season (20.59 ± 3.308). In the 6D group there was no significant difference between mid-season (23.44 ± 7.644) and post-season (24.61 ± 6.908). Data of 9ND showed no significant difference between mid-season (19.96 ± 2.723) and post-season (17.91 ± 3.130). In the mid-season, there was no significant difference between 6D (23.44 ±7.644) and 9ND (19.96 ± 2.723). In the post-

season, there was no significant difference between 6D (24.61 ± 6.908) and 9ND (17.91 ± 3.3130).

Nitrotyrosine analysis detected no significant difference in mean concentrations between mid-season (131.3 nM ± 12.31) and post-season (151.6 ± 12.60). There were no significant differences in 6D between mid-season (152.4 ± 23.46) and post-season (151.9 ± 17.72) nor in 9ND between mid-season (118.2 ± 12.72) and post-season (151.4 ± 18.18). Nitrotyrosine concentrations between groups revealed no significant differences between 6D (152.4 ± 23.46) and 9ND (118.2 ± 12.72) in mid-season nor between 6D (151.6 ± 12.60) and 9ND (151.4 ± 18.18) in the post-season.

Analysis of SOD revealed significantly lower activity in mid-season (0.02065 U/mL ± 0.006656) than post-season (0.04459 ± 0.005860) (Figure 3.2). Among 6D, there was significantly lower SOD activity in mid-season (0.01241 ± 0.006469) than post-season (0.05048 ± 0.004688) (Figure 3.3). Analysis of 9ND exhibited no significant difference between mid-season (0.02385 ± 0.009359) activity and post-season (0.04326 ± 0.009059). In mid-season, 6D (0.01241 ± 0.006469) and 9ND (0.02385 ± 0.009359) displayed no significant difference. In post-season, no significant difference was found between 6D (0.05048 ± 0.004688) and 9ND (0.04326 ± 0.009059).

Discussion

Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is ubiquitous with human life [10]. Numerous mechanisms, both endogenous and exogenous, contribute to ROS and RNS production. Primary sources of endogenous ROS include mitochondria [11], peroxisomes, endoplasmic reticulum, and phagocytic cells [12]. Exogenous

sources include air pollution, radiation, alcohol, tobacco, heavy metals, transition metals, pesticides, industrial solvents, and certain drugs such as halothane (anesthetic) and acetaminophen (analgesic/fever reducer) [12]. Relative to exercise, mitochondrial respiration is the most important production mechanism for free radicals. Electrons "leak" from the electron transport chain via semiquinone anion, namely in complex I and complex III, and join molecular oxygen to form superoxide ion radical [12]. The non-enzymatic nature of ROS production through mitochondrial respiration means that at a higher metabolic rate more ROS is produced [12]. A "crucial balance" between ROS and RNS production and antioxidant defense may play an important role in endogenous disease prevention [10]. Oxidative stress from exercise and/or environmental toxins can cause a disruption in this crucial balance.

A healthy system will overcome oxidative stress through endogenous and exogenous antioxidants. A normal return to homeostasis typically occurs with isolated or short-term oxidative stress. For example, regular bouts of moderate exercise will initially increase ROS and RNS. Homeostasis will be achieved, however, if the body adjusts to greater demands on mitochondrial respiration. As such, the "crucial balance" will be reclaimed. However, if oxidative stress outstrips the body's ability to adjust, by frequency and/or magnitude, disease may follow. In the case of endurance athletes, OTS may result [1]. ROS and RNS induced by oxidative stress have been linked to diabetes mellitus (DM), neurodegenerative diseases, cancer, cardiovascular diseases (CVD), cataract lenses, rheumatoid arthritis, and asthma [12]. Abnormal inflammation, a condition associated with a series of risk factors for metabolic diseases including DM, CVD, obesity [13], and rhabdomyolysis [14], is closely associated with oxidative stress and is a central pillar of the cytokine theory of OTS [1].

Lipid peroxidation is characterized by the oxidation of lipid molecules by free radicals. Polyunsaturated fatty acid residues of phospholipids in cellular lipid membranes are particularly vulnerable to peroxidation [12]. Through a cascade of events including the formation of a lipid radical ($L^{\bullet}$), then a peroxy radical ($LOO^{\bullet}$), the primary product of lipid peroxidation is formed, lipid hydroperoxides (LOOH). In addition to LOOH, several secondary products are formed including HNE, "…a major bioactive marker of lipid peroxidation…" [15]. Our data indicate that the group of participants who experienced at least one decline in athletic performance lasting at least three weeks during the competitive season had significantly higher mean HNE concentration than those who did not experience such a decline (Figure 3.1). Higher HNE concentration suggests a higher degree of lipid peroxidation and cellular damage in the 6D group than the 9ND group even after a one-month recovery period.

A vasodilator produced in epithelial cells, NOX reacts quickly with superoxide anion ($O_2^{\bullet-}$) to form a powerful and toxic oxidant, peroxynitrite ($ONOO^-$), continuing the oxidative cascade [16]. While NOX plays a critical role in mitochondrial respiration, excess NOX can mediate oxidative damage and may downregulate energy production [17]. Due to the short half-life of NOX, quantification of NOX in samples is often performed by measuring its final products, NO_2^- and NO_3^-. NOX is the only known biological molecule that, at high enough concentration, can outcompete endogenous SOD for $O_2^{\bullet-}$. We found no significant differences in NO_2^- and NO_3^- concentration in any of our comparisons suggesting that endogenous and exogenous $O_2^{\bullet-}$ scavengers may have outpaced $ONOO^-$ production.

Interaction between the amino acid tyrosine and $ONOO^-$ yields nitrotyrosine, a marker of nitrative stress and inflammation [18]. Protein-bound nitrotyrosine is present in a wide range of diseases with an inflammatory element including cardiovascular disease and diabetes [19]. Our

data revealed no significant difference in mean concentration of nitrotyrosine between mid-season and post-season conditions for the entire group, 6D, and 9ND. No significant differences were found between 6D and 9ND in either condition. These findings suggest that oxidative protein damage may not have exceeded the ability to mitigate such damage.

$O_2^{\bullet-}$ is toxic and, unless an imbalance exists, is dismutated immediately by SOD to form hydrogen peroxide (H_2O_2) which, in turn, is reduced to H_2O by catalases, glutathione peroxidases, and peroxiredoxins [20]. We tested for SOD activity as it "…is a first line of defense against toxicity of superoxide anion radicals" [20]. SOD from plasma was used as an oxidative stress biomarker although this may not actually reflect exact oxidation rates at the cellular level. Plasma levels are an accepted marker for oxidative stress analysis [9, 21]. Total SOD activity was measured which included for all three SOD metalloenzymes: cytosolic (SOD 1), mitochondrial (SOD 2), and extracellular (SOD 3). Mean SOD activity was significantly lower in mid-season as compared to post-season (Figure 3.2). For the group of participants who reported a drop in athletic performance, mean SOD activity was also significantly lower in mid-season as compared to post-season (Figure 3.3). For the overall group, SOD activity doubled in the post-season while for the 6D group the activity increased by a factor of four. The SOD activity for the entire group and for 6D contrasts with the mean SOD activity for 9ND which was not significantly different between mid-season and post-season. The 6D group was driving the mean SOD activity values and statistical significance for the entire group.

While it is clear that SOD activity for 6D was higher in the post-season recovery condition, there are several possible explanations. Higher SOD activity in 6D may indicate that a greater increase in $O_2^{\bullet-}$ production has occurred. Typically, increased production of $O_2^{\bullet-}$ takes place during exercise or periods of illness. All six participants of the 6D group reported experiencing

at least one drop in performance lasting three weeks or longer during the competitive cross country ski season. It is unclear if the drop in performance is the only factor that accounts for the higher SOD activity in the post-season. Muscle damage and associated inflammation could potentially explain the increase in endogenous plasma antioxidant activity. Why SOD activity increased in the post-season and not in the middle of the competition season when stress levels, presumably, were higher would need explanation. What is clear from the SOD activity data is that members of the 6D group had something from which to recover. More extensive physical and biochemical examination would be beneficial during the season.

The SOD activity meshes somewhat with HNE data. In the post-season recovery condition, the 6D mean HNE concentration is over twice as high as the 9ND mean and significantly different (Figure 3.1). Higher SOD activity suggests that participants in the 6D group may have been experiencing higher lipid oxidation than participants in the 9ND group in the post-season recovery condition. The SOD activity discrepancy would support the notion that SOD activity was higher in 6D in the post-season due to oxidative stress. It is problematic, however, that there were no other significant differences in mean HNE concentrations in any of the other four comparisons. The sensitivity and relatively high level of standard error associated with each mean may explain at least part of this question. There are no extensive studies showing that the biomarkers actually correlate. It is likely that a larger sample size would clarify the seeming inconsistency; however, these larger studies present logistical challenges.

Our study identified six participants who displayed at least one athletic performance symptom associated with OTS. Samples from the six participants revealed higher SOD activity in the post season. The samples also displayed higher HNE concentration as compared to 9ND in the post-season, which is consistent with oxidative stress for the 6D group. This study

provides additional support to the oxidative stress hypothesis of OTS. It also suggests that athletes may benefit from regular monitoring of markers of oxidative stress. A more robust sample size with a project design that includes quarterly sampling for an entire year should advance the understanding of the links between oxidative stress and athletic performance.

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Conflicts of Interest

The authors have no conflicts of interest to report. The lead author had coached some of the volunteer participants a few years prior to the commencement of this study. The lead author was not coaching at the time of participant recruitment nor during any part of the study. In addition, the lead author was not involved in the administration of the cognitive function tests. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Table 3.1 Participant Characteristics

	N	Age	Body fat (%)		Total MET† (min/wk)	
			Mid-season	Post-season	Mid-season	Post-season
Total	15	20.4 ± 1.64	NA	NA	5335 ± 427	3185** ± 446
Male	8	20.6 ± 1.92	12.0 ± 0.387	11.9 ± 0.573	4903 ± 449	3447 ± 735
Female	7	20.1 ± 1.35	29.4 ± 2.22	28.7 ± 2.29	5830 ± 754	2886* ± 495

Age, percent body fat, and MET minutes/week for all participants, males, and females. Mean values ± SEM. †Metabolic Equivalent Task. **$p<0.01$ *$p<0.05$

Figure Captions

Figure 3.1. Post-Season Plasma HNE. In the post-season recovery condition, mean plasma HNE concentration was significantly higher ($p<0.05$) for participants who reported at least one unexplainable drop in athletic performance lasting three weeks or longer during the competition season (6D) (197.5 μg/mL ± 22.79) than for participants who did not report such a drop (9ND) (69.80 μg/mL ± 33.59).

Figure 3.2. Comparison of Superoxide Dismutase Mid-Season and Post-Season. Participants displayed significantly higher ($p<0.05$) SOD activity in the post-season recovery condition (0.02065 U/mL ± 0.006477) as compared to the mid-season condition (0.04459 U/mL ± 0.005860).

Figure 3.3. Comparison of Superoxide Dismutase of Athletes Reporting a Drop in Performance. Mean SOD activity for participants who reported at least one unexplainable drop in athletic performance lasting three weeks or longer during the competition season (6D) was significantly higher ($p<0.05$) in the post-season condition (0.05048 U/mL ± 0.004688) than in the mid-season condition (0.01241 U/mL ± 0.006469).

Figure 3.1

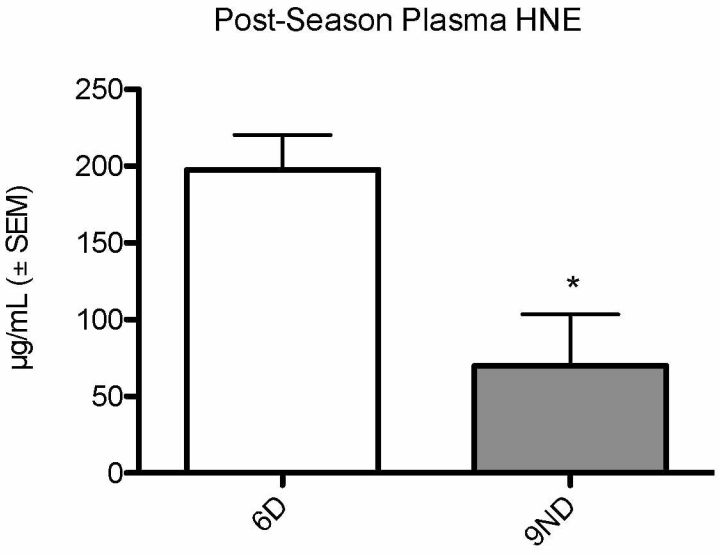


Figure 3.2

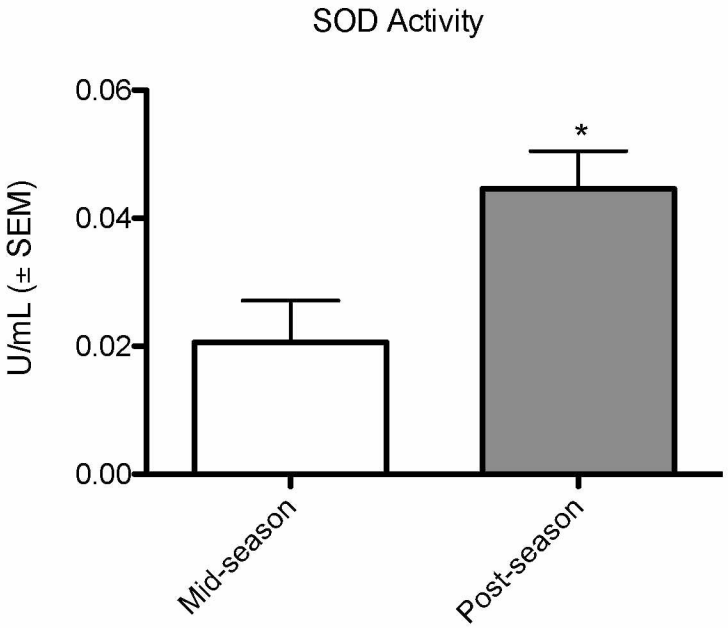
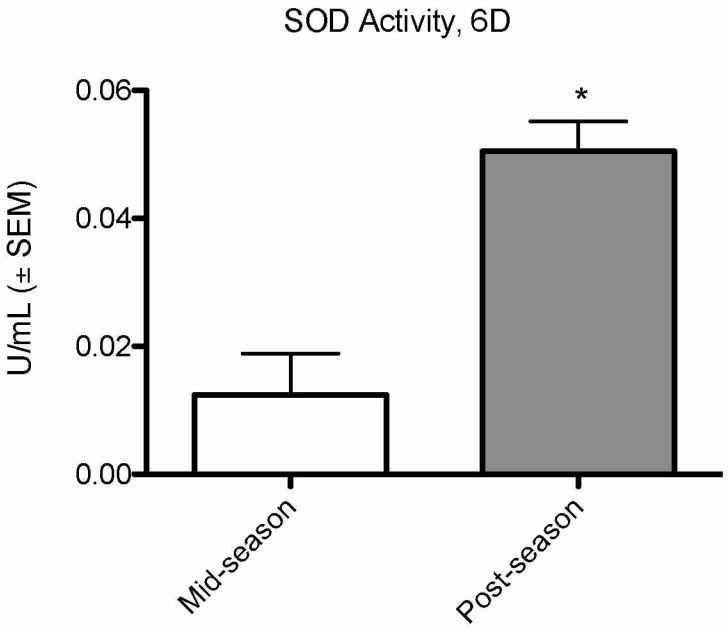


Figure 3.3


SOD Activity, 6D
U/mL (± SEM)
0.06
0.04
0.02
0.00
*
Mid-season
Post-season

General Conclusion/Future Directions

The studies herein revealed several clues that may provide valuable insight into the etiology of overtraining syndrome (OTS). Collegiate endurance athletes are more likely to be vitamin D insufficient or deficient than their sedentary counterparts. Collegiate cross country ski racers in the circumpolar North are unlikely to maintain even a minimum level of vitamin D adequacy during the winter's competition season. Furthermore, vitamin D levels of these skiers are likely to drop in the post-season, recovery period despite higher levels of sunlight. Cognitive function is significantly higher in the post-season than during the competition season. Finally, those who experienced a drop in performance during the competition season are more likely to show signs of oxidative stress. Despite these interesting findings it remains unclear if any of these are causes of, or effects from, OTS. Regardless, the association of some of these markers with underperformance could help create a screening profile to determine which athletes are at the greatest risk for developing OTS. At the very least, the results of these collective manuscripts have revealed biomarkers of interest that warrant further investigation.

In order to investigate further, I propose two research projects. The first uses a unique athlete model for markers associated with OTS. Sprint and distance sled dogs have been recognized as some of the top athletes in the world regardless of species. With a maximum oxygen uptake ($\dot{V}O_2$ max) approaching 240 mL/kg/min [1] and the ability to run at a speed of approximately 32 km/h (20 mph) for up to 75 minutes (+/-) per session, it is not hyperbole to say that the sprint-distance sled dog may be the fittest animal ever tested for aerobic power. The fittest human athletes do not even begin to approach the canine level of oxygen consumption. At the elite level, cross country skiers are considered to be among the fittest athletes. Medal

winners at events such as the Winter Olympic Games and World Championships have $\dot{V}O_2$ max levels of ~80-90 mL/kg/min for men and approaching ~75 mL/kg/min for women [2].

Distance sled dogs, those competing in events such as the 1,600 km (1,000 mi) Iditarod Trail Sled Dog Race, are bred for extreme weather conditions, longer runs, and multiple days of running. Distance dogs run at a slower pace than sprint dogs, though they run for hours and hours, day after day, whereas most sprint competitions are two or three days events with 15-75 minutes of effort per session. Distance dogs expend of over 11,000 kcal per day during a multi-day event [3], while an ultra-triathletes expend ~4200 kcal per day when completing five Ironman-distance triathlons in five consecutive days [4]. When adjusted for body mass, the distance dog expends approximately eight times more energy than the human during multi-stage, endurance races.

Most sled dogs from competitive teams are raised, trained, fed, and cared for in controlled conditions. Typically, dogs from one kennel – particularly those on the same team within a kennel – receive the same diet, are trained the same amount and in the same manner, and receive the same rest and recovery periods. Sled dogs do not have to cope with the same external factors as their human athlete counterparts. In addition to sports training, human athletes must contend with a variety of pressures including jobs, family dynamics, relationships, societal expectations, media, schoolwork, etc. These additional stressors add to the overall complexity of strain which may contribute to non-functional overreaching or overtraining. Sled dogs are also bred for conformational similarity to optimize performance. Thus, a high level of genetic homogeneity is achieved in a particular kennel. Competitive sled dogs, whether they be sprint dogs or distance dogs, are therefore uniquely suited to serve as a model for specific aspects of training and performance in human endurance athletes.

The notion that a researcher would be able to quantify and record complete dietary intake, health status, training regimen, and athletic performance over weeks and months in one single athlete is almost unheard of. Totalitarian states with unethical, elite sports programs notwithstanding, it would be practically impossible to reach this comprehensive level of monitoring for a group of 20-40 human athletes. This level of monitoring is, however, precisely what is possible by studying sled dogs from competitive racing kennels. With a high level of precision and accuracy, researchers are able to monitor the best endurance athletes in the world.

I propose that competitive sled dogs, from both sprint and distance kennels, be used for studying OTS. No dogs should ever be purposely overtrained. By monitoring dogs from the start of a new competitive cycle (May or June) through the completion of competition season (March or April) we may be able to identify those whose performance has dropped for no readily apparent reason. Dogs that have an injury or other condition (age, pregnancy, heat cycle, etc.) that explains underperformance would be excluded. Typically, an underperforming dog is "dropped" from the team and replaced by another. By examining biomarkers from all dogs from a kennel on a monthly basis, researchers may be able to identify differences, subtle or concrete, between dropped dogs and those performing "normally" or up to expectation. While the underperformance may not manifest itself until the racing season, clues as to why a dog reached that point may be available from data collected in the summer and fall. The proverbial "straw that broke the camel's back" may have little to do with the reason that a dog reached the point of underperformance and hence was dropped from a team. The answer is more likely to come from the months leading up to that circumstance. A sled dog study combining thorough record keeping and regular sampling may provide clues for further OTS research with human endurance athletes.

The second project is also longitudinal and requires human athletes as research participants. It is based on the same principle as the sled dog study. What is different between those human athletes whose performance improves over time and those who, despite training in an "appropriate" way, find their performance declining? While we would like to keep "all things equal" for athletes in the study, this concept is utterly impossible in practical terms. We can, however, monitor critical components to serve as markers for health, mental stress, physical stress, and performance.

I propose that we recruit 40 (20 = M, 20 = F) elite, cross country skiers from clubs around the world located at a similar latitude who are not experiencing a period of underperformance. We will sample and test athletes five times through the annual training cycle: 1) beginning of the new, training cycle (May 1); 2) following the summer training macrocycle (August 30); 3) prior to the racing season (November 1); 4) immediately following the competitive season (April 8); and 5) following the recovery period (April 30). Blood samples would be tested for vitamin D, inflammatory cytokines, markers of oxidative stress, and a general chemistry/health panel. The Purdue Pegboard Test and the Profile of Mood States test would be administered along with questionnaires regarding training load, health, and athletic performance. Finally, participants would complete a submaximal exercise test with a blood lactate profile to evaluate aerobic fitness.

We will establish baseline values as well as values for athletes progressing at a "normal" rate of performance improvement. We will compare baseline values and normal progression values with themselves over time as well as against participants who experience a plateau or drop in athletic performance and/or other symptoms associated with OTS. Longitudinal methodology along with a robust sample size will allow us to pinpoint abnormalities that may

have been present before the onset of OTS symptoms. This design will give us a better understanding of what tests to include in a pre-OTS screening panel. Information from the panel could then be used to help athletes and trainers alike make intelligent choices about training load, recovery periods, and overall lifestyle.

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1. Reynolds AJ, Reinhart GA, Carey DP, Simmerman DA, Frank DA, Kallfelz FA. Effect of protein intake during training on biochemical and performance variables in sled dogs. Am J Vet Res. 1999;60:789-95.

2. Holmberg HC. The elite cross-country skier provides unique insights into human exercise physiology. Scand J Med Sci Sports. 2015;25 Suppl 4:100-9.

3. Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA. Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold. Am J Vet Res. 1997;58:1457-62.

4. Knechtle B, Knechtle P, Andonie JL, Kohler G. Body composition, energy, and fluid turnover in a five-day multistage ultratriathlon: a case study. Res Sports Med. 2009;17:104-20.

Appendix A

(907) 474-7800
(907) 474-5444 fax
uaf-irb@alaska.edu
www.uaf.edu/irb

Institutional Review Board
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

November 15, 2016

To:	Arleigh Reynolds, DVM, PhD Principal Investigator
From:	University of Alaska Fairbanks IRB
Re:	[838437-5] IGF-1, MCP-1, 25OHD, and mental states: An OTS pilot study

Thank you for submitting the Continuing Review/Progress Report referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	IGF-1, MCP-1, 25OHD, and mental states: An OTS pilot study
Received:	November 11, 2016
Expedited Category:	*2 and 7*
Action:	APPROVED
Effective Date:	November 15, 2016
Expiration Date:	December 10, 2017

This action is included on the December 14, 2016 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.

Appendix B

(907) 474-7800
(907) 474-5444 fax
uaf-irb@alaska.edu
www.uaf.edu/irb

Institutional Review Board
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

January 27, 2016

To:	Arleigh Reynolds, DVM, PhD Principal Investigator
From:	University of Alaska Fairbanks IRB
Re:	[838437-3] IGF-1, MCP-1, 25OHD, and mental states: An OTS pilot study

Thank you for submitting the Amendment/Modification referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	IGF-1, MCP-1, 25OHD, and mental states: An OTS pilot study
Received:	January 26, 2016
Expedited Category:	*2 and 7*
Action:	APPROVED
Effective Date:	January 27, 2016
Expiration Date:	December 10, 2016

This action is included on the February 3, 2016 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.

Appendix C

(907) 474-7800
(907) 474-5444 fax
fyirb@uaf.edu
www.uaf.edu/irb

Institutional Review Board
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

August 7, 2013

To: Kriya Dunlap, PhD
Principal Investigator

From: University of Alaska Fairbanks IRB

Re: [492213-2] A pilot study: GLUT-4 and conditioning

Thank you for submitting the Amendment/Modification referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	A pilot study: GLUT-4 and conditioning
Received:	August 7, 2013
Expedited Category:	*2*
Action:	APPROVED
Effective Date:	August 7, 2013
Expiration Date:	August 7, 2014

Required Information:

Minor modifications were requested and have been reviewed administratively.

This action is included on the September 11, 2013 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.

Appendix D

(907) 474-7800
(907) 474-5444 fax
fyirb@uaf.edu
www.uaf.edu/irb

Institutional Review Board

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

September 30, 2013

To:	Kriya Dunlap, PhD Principal Investigator
From:	University of Alaska Fairbanks IRB
Re:	[492213-4] A pilot study: GLUT-4 and conditioning

Thank you for submitting the Amendment/Modification referenced below. The submission was handled by Administrative Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	A pilot study: GLUT-4 and conditioning
Received:	September 30, 2013
Expedited Category:	*2 and 7*
Action:	APPROVED
Effective Date:	September 30, 2013
Expiration Date:	August 7, 2014

Required Information:

Minor modification was made and administratively approved as requested by reviewer in package 3 (previous package).

This action is included on the October 2, 2013 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.